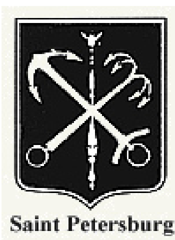
Достижения ГЕНОМИКИ - МЕДИЦИНЕ. Отчет о поездки в Амстердам 28 -31 мая 2011 г.(ESHG).

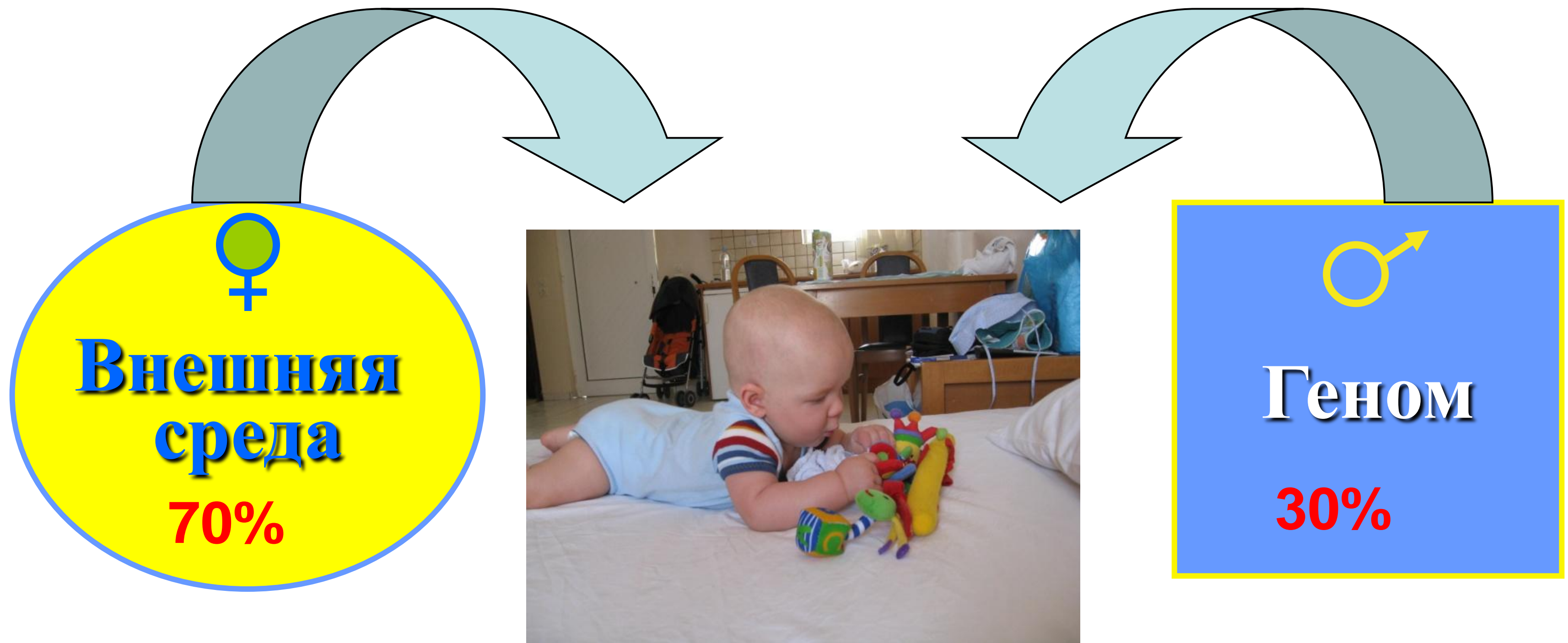


Глотов О.С.

НИИ АГ им. Д.О.Отта СЗО РАМН Санкт-Петербург,

Санкт-Петербург, 2011г

СЛАГАЕМЫЕ ЗДОРОВЬЯ



ШЕСТЬ ОСНОВНЫХ ФАКТОРОВ, ВЛИЯЮЩИХ НА ЭКСПРЕССИЮ ГЕНОВ

ЕДА, РЕЖИМ ПИТАНИЯ, ФИЗИЧЕСКАЯ АКТИВНОСТЬ, СТРЕСС ВРЕДНЫЕ ПРИВЫЧКИ, ЭКОЛОГИЯ, ЛЕКАРСТВА

Глотов О.С., 2001

Основные направления молекулярно-генетических исследований

Диагностика моногенных болезней

Пренатальная диагностика

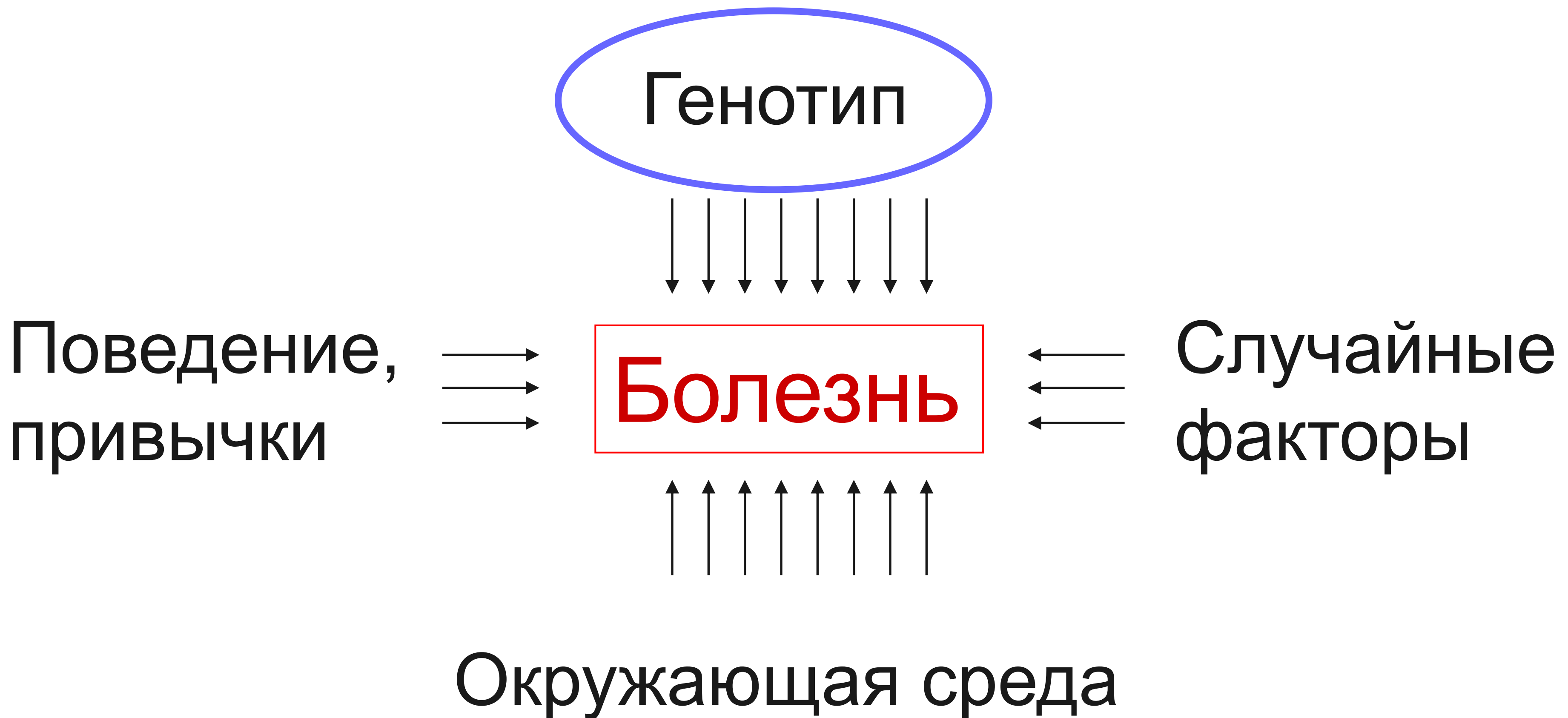
Изучение мультифакториальных заболеваний

Предиктивное тестирование

Популяционные исследования (GWAS)

Глотов О.С., 2001

Мультифакториальные (комплексные) заболевания



Глотов О.С., 2001

ГЕНЫ «ПРЕДРАСПОЛОЖЕННОСТИ» -

«мутантные» гены, наличие которых совместимо с жизнью человека, но которые в неблагоприятных условиях могут быть причиной различных заболеваний

ГЕНЫ «ПРЕДРАСПОЛОЖЕННОСТИ» -

варианты генов, которые совместимы с жизнью, но в неблагоприятных условиях могут провоцировать развитие различных заболеваний

- Гены системы детоксикации ксенобиотиков
- Гены рецепторов
- Гены - метаболические шунты
- Гены «старения»
- Гены иммунной защиты
- Генные сети мультифакториальных заболеваний и др.

ЗНАЧЕНИЕ ГЕНЕТИЧЕСКОГО ТЕСТИРОВАНИЯ

- *Выделение **групп риска***
- *Внесение корректировок в **терапию***
- ***Коррекция** образа **жизни и тренировочного процесса***

ГЕНЕТИЧЕСКОЕ ТЕСТИРОВАНИЕ – ЭТО:

-Метод прямого выявления мутации
-Не зависит от функционального состояния организма
-На основании полученных данных можно сформировать план обследования и профилактических мер

Глотов О.С., 2001

ГЛАВНЫЕ ПРОБЛЕМЫ ПРЕДИКТИВНОЙ МЕДИЦИНЫ

1. Достоверность результатов генетического тестирования наследственной предрасположенности

2. Выявление всех генов , ассоциированных с конкретным МФЗ

3. Адекватная интерпретация результатов

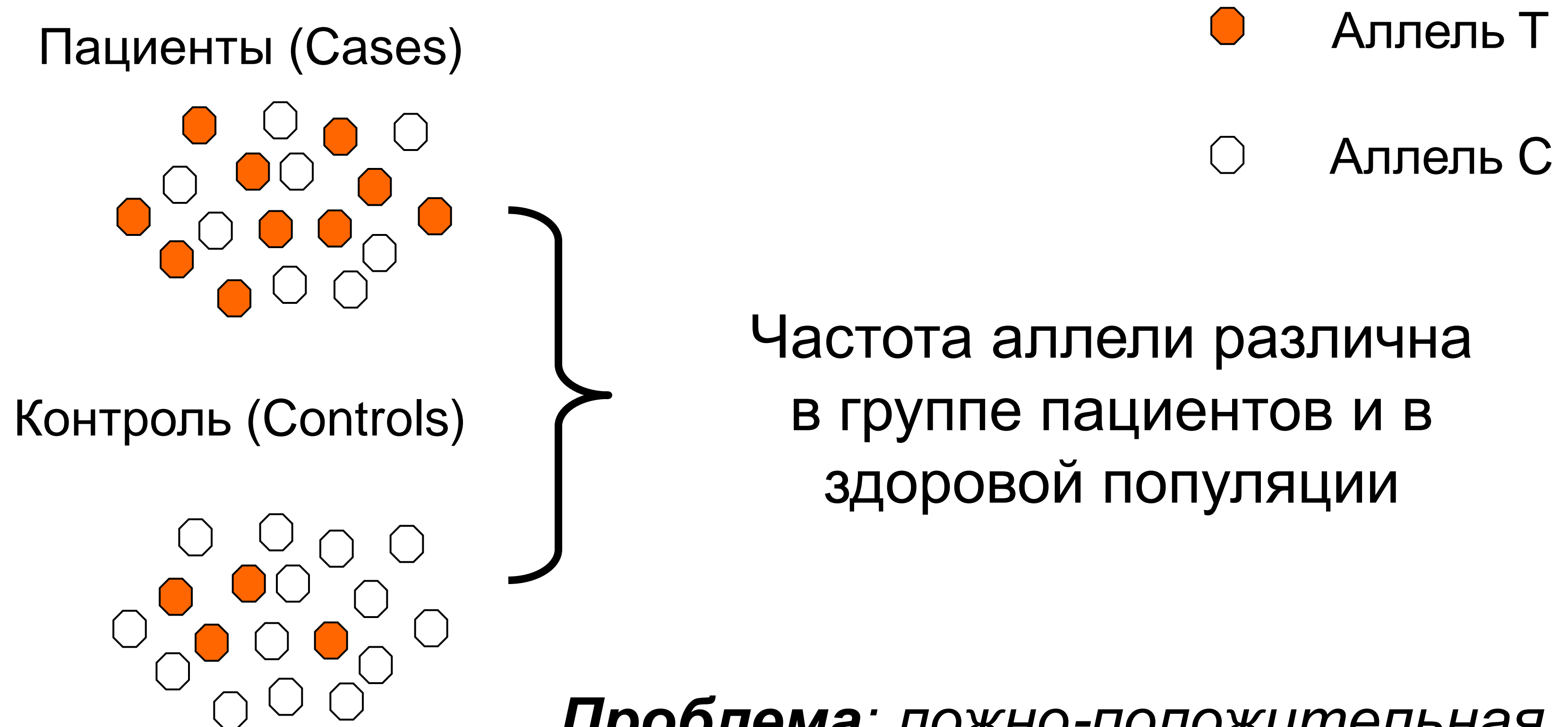
Глотов О.С., 2001

Исследования генов-кандидатов

- Гипотеза -> генетические исследования
- Анализ ассоциации
- Точечные полиморфизмы (SNPs)
- До недавнего времени – органичивались тестированием 1-2 функциональных вариантов (nsSNPs).
- После выхода в свет HapMap – вместо функциональных вариантов стали типировать ключевые полиморфизмы (tagSNPs) (максимальное «покрытие» гена-кандидата)

Анализ ассоциации - методика (1)

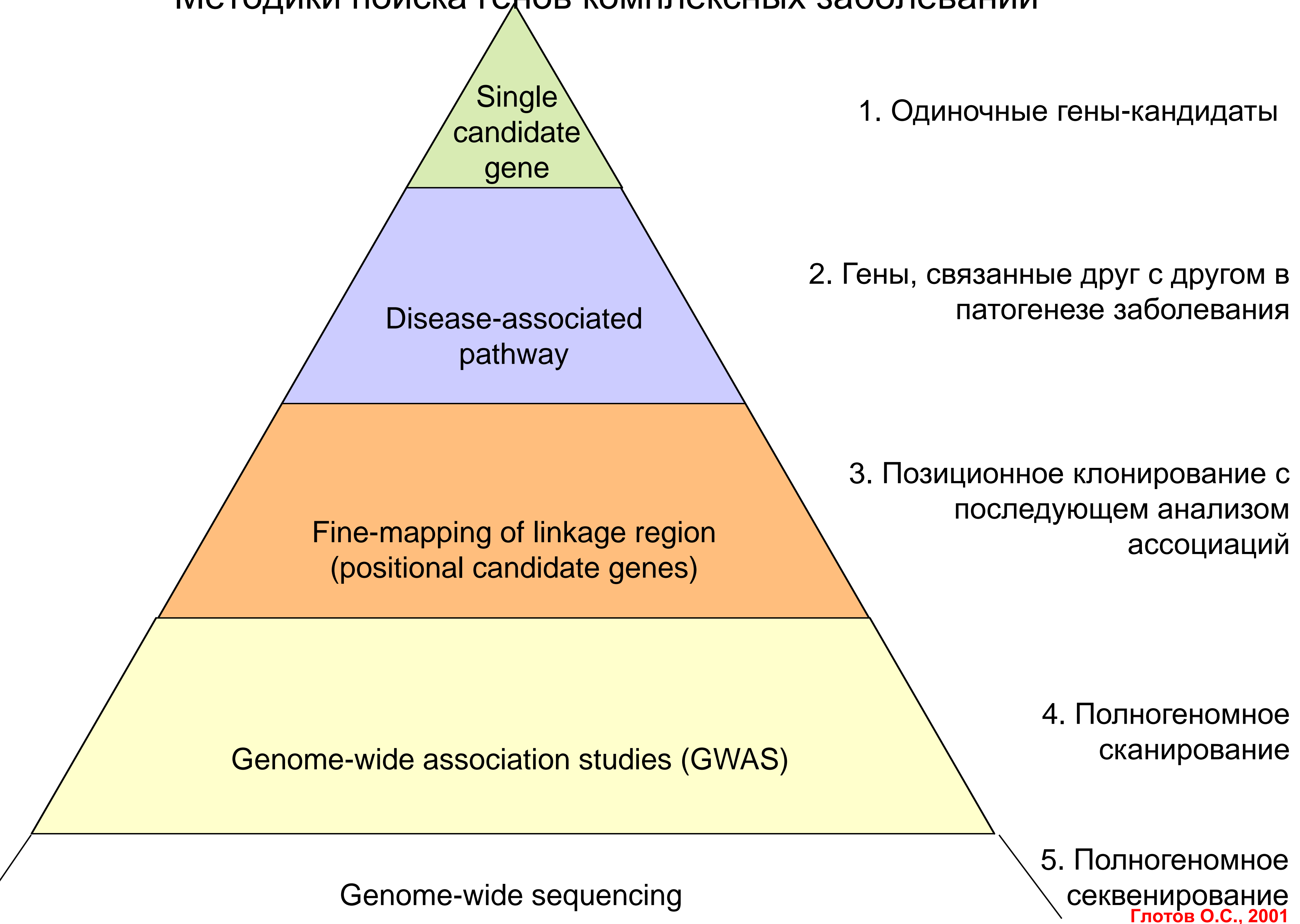
Популяционное исследование: case-control association studies



***Проблема**: ложно-положительная ассоциация из-за различий между пациентами и контрольной группой, не связанных с заболеванием*

Глотов О.С., 2001

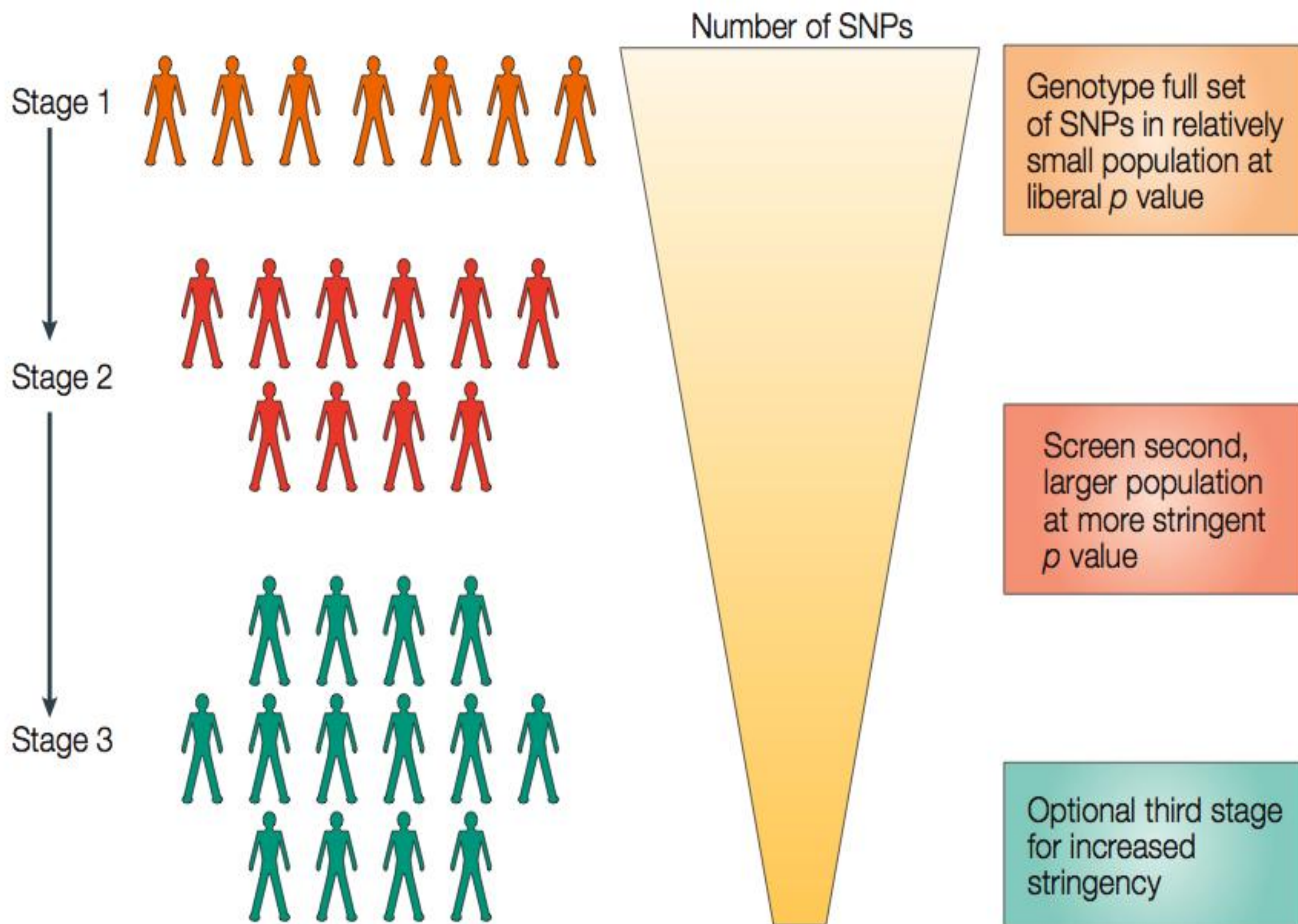
Методики поиска генов комплексных заболеваний



Сканирование генома (GWAS)

- Genome-wide association study (GWAS)
- Анализ ассоциации. Используется большое количество маркеров на всех хромосомах.
- Две основные конкурирующие фирмы (поставщики оборудования) – Illumina и Affymetrix
- Первый GWAS в целиакии (Англия) – *Illumina 317K* (317,000 SNPs)
- Текущие исследования – GWAS в Голландии, Италии, Финляндии и Англии – *Illumina 670 quad bead chip* (540,000 SNPs + CNV (copy-number variations – области делеций и дупликаций)

Методика полногеномного сканирования



Глотов О.С., 2001

European Human Genetic Conference 2009,Vienna

Семинар : **GWAS or not to GWAS?**

Основные средства научных программ в рамках (GEN2PHEN) и коммерческих фирм, проводящих генетическое тестирование, следует тратить на выяснение клинической значимости результатов генетического тестирования наследственной предрасположенности

Джордж Черч

КАЖДОМУ — по геному!

43-я ежегодная конференция по генетике человека (43th European Human Genetics Conference, ESHG 2011) проходила в городе Амстердам, Нидерланды с 28 мая по 31 мая 2011 года.

Хочется отметить значительный размах проводимого мероприятия: количество зарегистрированных участников составило более 2200 человек (2129 постерных докладов) из Европы, Америки, Японии, Китая и других регионов мира (к сравнению в 1989 году было 350 участников и 185 постерных докладов). Пленарные лекции читали ведущие мировые ученые.

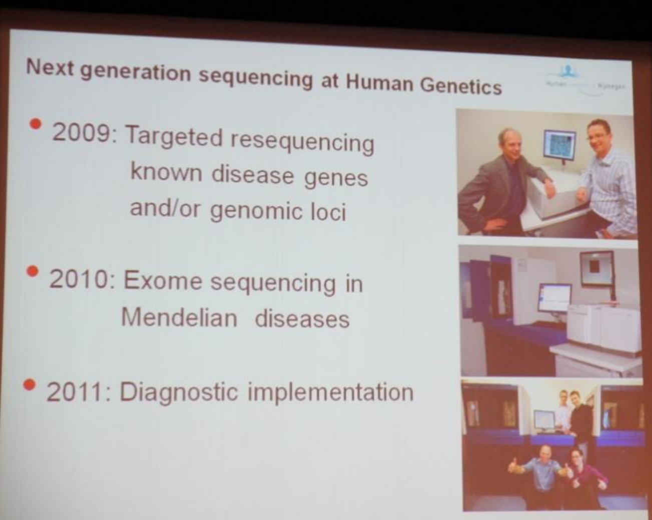
Чипы нового поколения позволяют изучать тысячи генов одновременно (так называемый EXOM). С помощью данного метода мы можем расшифровать всю кодирующую последовательность изучаемых генов за несколько часов. Сейчас такие исследования проводят в основном для поиска мутаций у пациентов с редкими заболеваниями.

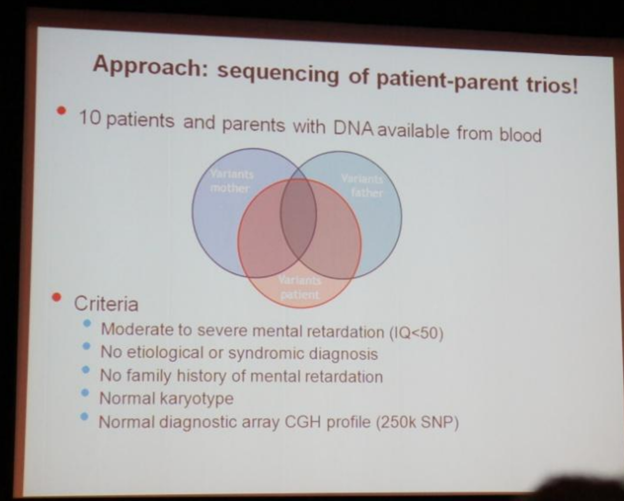
Для тех кто хочет публиковаться в Европейском журнале по генетике человека привожу их IF.

European Journal of Human Genetics

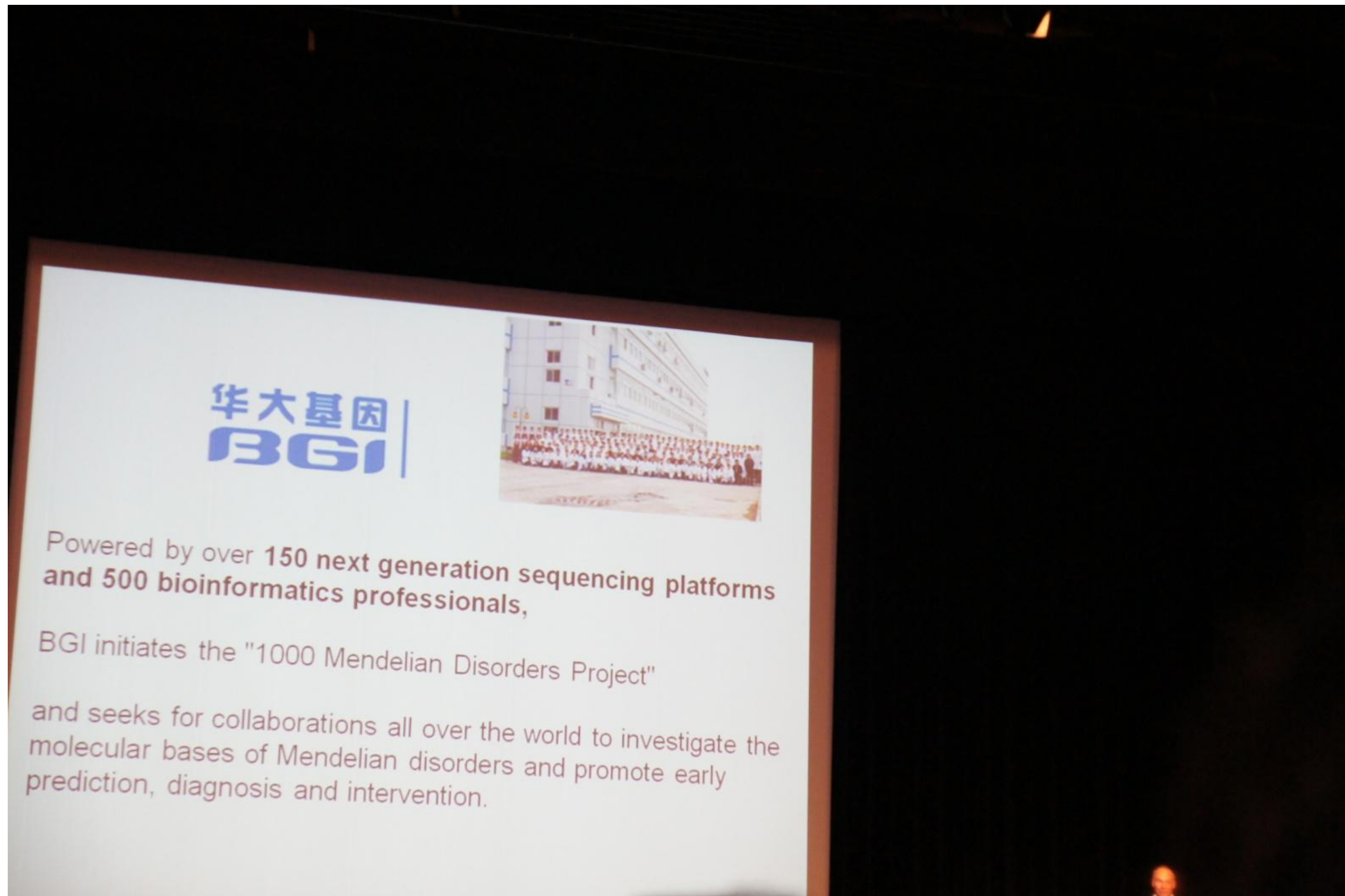
IF = 3.564

Важно подчеркнуть, что особый акцент исследований сосредоточен на анализе генетического материала с помощью биочипов нового поколения, позволяющего в краткое время получить полную кодирующую нуклеотидную последовательность генома человека в течении 2-3 часов (Приборы фирм Life technologies и Roche).





Причем большинство полногеномных исследований сейчас проводятся в Китае!!!



Unraveling the Dutch genome in health and disease

C. Wijmenga;

Faculty of Medical Sciences, Department of Medical Genetics, University Medical Center Groningen, Groningen, Netherlands.

Genome of the Netherlands (GoNL)

- Aim: To establish a map of Dutch genetic variation by whole genome-sequencing of 1000 independent Dutch genomes
- Unique family-based design: 250 trios
  - 230 x 2 parents – 1 offspring
  - 10 x 2 parents – 2 offspring
  - 10 x 2 parents – 1 MZ twin offspring

- Advantages compared to 1000 Genomes Project:
  - Phase information; accurate haplotypes
  - Better characterization of Structural Variation
  - Detection of *de novo* variants and new mutation rates
- Specifications:
  - Families equally distributed over the Dutch provinces
  - Genomic DNA, paired-end sequencing on HiSeq2000, 12x coverage
  - Genotyped on immunochip for QC purposes

GoNL consists of 3 phases

Sequence analysis

BGI Premier Scientific Partner

Sequencing 770 genomes completed:
- 230 trio's (690)
- 10 quartets (40)
- 10 parent pairs with MZ twin offspring (40)

Data analysis & Method development

- ~30% of data aligned to reference (hg19)
- In-depth analysis on 20 trio's (pilot)

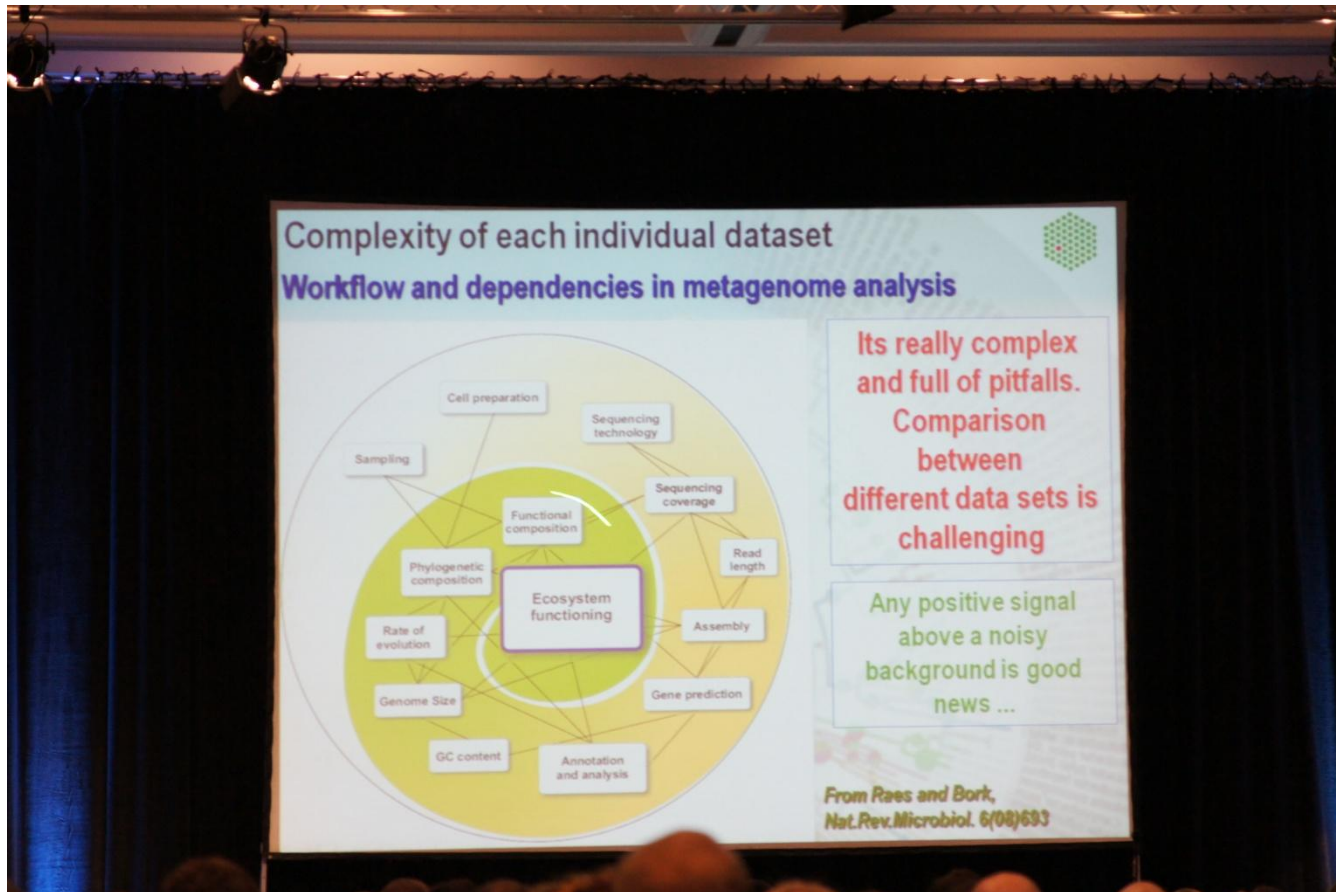
Imputation existing GWAS

~100,000 Dutch samples with GWAS data

Exploring the invisible world using metagenomics: systemic analysis of the ecosystem ‚human gut‘

P. Bork;

Structural and Computational Biology, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.



Глотов О.С., 2001

Sequencing Thousands of Human Genomes

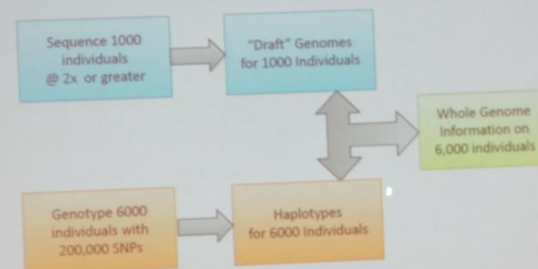
G. Abecasis;

Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI, United States.

SardiNIA Whole Genome Sequencing

- Francesco Cucca, David Schlessinger, Gonçalo Abecasis
- 6,148 Sardinians from 4 towns in Ogliastra
  - Recruited among population of ~9,841 individuals
  - Sample includes >34,000 relative pairs
- Measured ~100 aging related quantitative traits
- Whole genome sequencing
  - Plan to sequence >1,000 individuals at 2x
  - ~200,000 SNPs genotyped in all individuals
  - >500,000 SNPs genotyped in ~2,500 individuals
- NHGRI/NIMH RC2 Medical Sequencing Project

Design



Глотов О.С., 2001

Impact of polygenic profile to the performance of endurance and strength/power athletes

[1,2]Valentina Ginevičienė, [1]Audronė Jakaitienė, [2]Linas Tubelis, [1]Vaidutis Kučinskas
[1]Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University;
[2]Lithuanian Olympic Sports Centre, Vilnius
E-mail valentina.gineviciene@gmail.com

INTRODUCTION

Human physical capability is influenced by many environmental and genetic factors, and it is accepted that physical capability phenotypes are highly polygenic.

To date, over 200 DNA polymorphisms have been associated with some form of human physical performance or a health-related fitness phenotype. For many of the polymorphisms associated with human performance, there has only been a single positive association with a relevant phenotype. Notable exceptions to this statement include the polymorphisms of the *ACE* (angiotensin I-converting enzyme), *ACTN3* (actinin, α3), *PPARGC1A* (peroxisome proliferative activated receptor, gamma, coactivator 1, alpha), *PPARA* (peroxisome proliferative activated receptor, alpha), *PPARG* (peroxisome proliferative activated receptor, gamma), *ADRB2* (adrenergic, β-2-, receptor), *AMPD1* (adenosine monophosphate deaminase 1), *APOE* (apolipoprotein E) and *BDKRB2* (bradykinin receptor B2) genes that have been studied by several research groups, using a variety of experimental designs and population types.

We investigated five genetic polymorphisms that are candidates explaining individual variations in human physical performance phenotypic traits (*ACE* I/D (rs1799752); *ACTN3* C/T (rs1815739); *PPARGC1A* G/A (rs8192678); *PPARA* G/C (rs4253778); *PPARG* C/G (rs1801282)) in professional Lithuanian athletes (n=193) and in the general population of Lithuania (nonathletic controls, n=250).

The athletes were prospectively stratified into two groups according to the event duration and distance, spanning a spectrum from the endurance-oriented to the power-oriented.

METHODS

According to Williams and Folland (*J Physiol* 586:113-121, 2008) we calculated the 'total genotype score'

$$TGS = \frac{100}{2k} \cdot (GS_{ACE} + GS_{ACTN3} + GS_{PPARGC1A} + GS_{PPARA} + GS_{PPARG})$$

(TGS, the combination of five polymorphisms with the maximum value of '100' for the theoretically optimal polygenic score, *k* number of the polymorphisms) in the athlete groups and in the Lithuanian population; and the probability for the occurrence of Lithuanian individuals with the 'perfect' polygenic physical performance phenotype (power-oriented and endurance-oriented) profile. The TGS was calculated for the "power" and "endurance" groups of the Lithuanian athletes.

DNA of the Lithuanian athletes was extracted from peripheral blood leukocytes by using phenol-chlorophorm method. The DNA fragment researched was amplified by using polymerase chain reaction (PCR) method. Single nucleotide polymorphisms (*ACTN3* C/T, *PPARGC1A* G/A, *PPARA* G/C, *PPARG* C/G) were analyzed by using restriction-fragment length polymorphism (PCR-RFLP) method. *ACE* I/D polymorphism analysis was performed using PCR method.

RESULTS

The frequency distributions of the genotypes of the candidate gene markers in the Lithuanian athletes and general population had a specific pattern.

We found the mean TGS significantly higher for the elite power-oriented athletes (44.4±11.3) compared to controls (33.6±13.2) (p<0.05) indicating more favorable polygenic profile for power-oriented athletes. No significant differences were found comparing the athletes in the endurance group (65.7±13.9) and controls (66.4±13.2). A single athlete had an "ideal" genotypic combination for endurance (TGS=100).

The obtained probability of a Lithuanian individual possessing a theoretically optimal endurance-oriented polygenic profile for up to five candidate genetic polymorphisms, equals to 1% (or 1 among 99 Lithuanian individuals); the optimal power-oriented polygenic profile accordingly 0.0007% (or 1 among 132650 Lithuanian individuals).

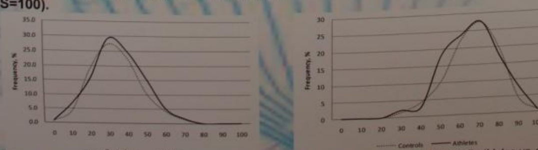


Figure 1. TGS distribution for power-oriented (left-sided) and endurance-oriented (right-sided) athletes *vs.* controls.

CONCLUSION

We have identified a polygenic profile that allows us to distinguish elite power athletes from nonathletic population. The optimal combination of genotypes considered may occur more frequently for endurance rather power among Lithuanian individuals.

European Human Genetics Conference 2011
Amsterdam RAI, The Netherlands, May 28 - 31, 2011

Глотов О.С., 2001

Association of *COMT* and *MTHFR* polymorphisms with cognition in schizophrenia

Dimitrios Kontis[1], Christalena Sofocleous[2,3], Eirini Theochari[1], Spyridon Kleisas[1], Emmanouel Kanavakis[2], Helen Fryssira[2]

[1] 1st Psychiatric Department, Psychiatric Hospital of Attica, Athens, Greece
[2] Department of Medical Genetics, Medical School, Athens University, "Aghia Sophia" Children's Hospital, Athens, Greece
[3] Research Institute for the Study of Mailgnant Disorders in Childhood, , "Aghia Sophia" Children's Hospital , Athens , Greece

Introduction

- Catechol-O-methyltransferase (COMT) contributes to enzymatic degradation of dopamine and noradrenaline. The human *COMT* gene on 22q11 contains a functional polymorphism (Val158Met, rs4680 G/A) affecting the activity of the enzyme at body temperature. Met 158 variant is supposed to exert availability of prefrontal dopamine signaling improving thus prefrontal activation during working memory performance.
- The *MTHFR* gene, coding for methylenetetrahydrofolate reductase which is implicated in gene regulation through methylation mechanisms, also contains a functional polymorphism (677C to T, rs1801133) that has been associated with overall schizophrenia risk and executive function impairment in patients.
- Recent findings revealed a possible epistatic interaction between *COMT* and *MTHFR* showing that patients who carried both the *COMT* Val variant and (low-methyl) *MTHFR* T alleles exhibited executive function deficits. Such an interactive contribution of *MTHFR* and *COMT* genotypes to cognitive dysfunction of prefrontal origin is of great importance especially for schizophrenic patients as it may be used to suggest potential targets for treatment.

Objectives

The investigation of the effect of *COMT* (Val108/158Met) and *MTHFR* (C677T) polymorphisms on the cognitive function in schizophrenia.

Material and Methods

- 92 patients with chronic schizophrenia (59 males, 33 females, mean age=42,92 yrs SD=9,92) and 61 healthy controls from the Psyciatric Hospital of Attica.
- **Genetic Analysis:** Polymerase Chain Reaction (PCR) was performed for the amplification of *COMT* and *MTHFR* regions of interest. Restriction Fragment Length Polymorphisms (RFLPs) analysis using Hinf I and Nla III restriction enzymes was followed for the detection of polymorphisms C677T and Val 158 Met on *MTHFR* and *COMT* genes respectively. Primers and conditions have been previously described in the literature.
- **Clinical evaluation:** The scale of Positive and Negative Syndrome in Schizophrenia (PANSS)
  The scale of General Interpretation of Functionality (GAF)
  The scale of Depression of Calgary (CDS)
- **Cognition evaluation:** Wechsler Adult Intelligence Scale-(WAIS-III) and the Cambridge Neuropsychological Test Automated Battery (CANTAB) were used . CANTAB tests measured: speed of movement (MOT), pattern and spatial recognition memory (PRM, SRM respectively), spatial working memory (SWM), planning (SOC) and cognitive flexibility (IEDSS).
- **Statistical Analysis** : ANOVA, [Kruskal Wallis (df=2)=6.828, p=0.033].

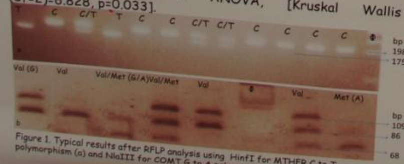


Figure 1. Typical results after RFLP analysis using HinfI for MTHFR C to T polymorphism (a) and NlaIII for COMT G to A polymorphism (b)

Results

Participants were divided into four groups according to their genetic polymorphisms (fig.1): [**VALC** (Val-Val & CC), **VALT** (Val-Val & CT or TT), **METC** (Val-Met or Met-Met CC), **METT** (Val-Met or Met-Met & CT or TT)]. Positive correlations of *COMT* and *MTHFR* polymorphisms to cognition were recorded in both the group of patients with schizophrenia and the control group (fig 2). Patients with the VAL/T combination presented higher full scale and performance IQ than patients with VAL/C (fig 3,4). In all patients a significant main effect of *COMT* in SOC was recorded, with those carrying VAL/C having a worse performance compared to the ones with MET/C. Finally, in respect to PRM, SRM and SWM controls carrying VALT performed better than those with VALC.

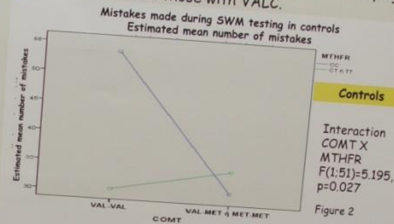


Figure 2

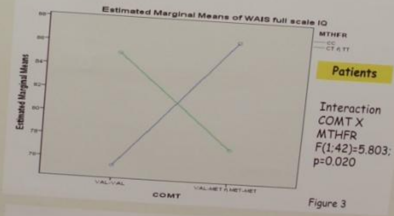


Figure 3

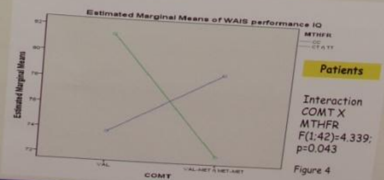


Figure 4

Conclusions

- There is strong evidence that supports the interaction between *COMT* and *MTHFR* genes on cognitive function in both the schizophrenic patients and the healthy population
- MTHFR T allele contributes to better cognitive function when combined with *COMT* Val homozygosity
- Our findings may prove to be valuable proposing possible therapeutic strategies towards cognitive dysfunction in schizophrenia based on both the dopaminergic and the methylation pathways

Глотов О.С., 2001

P09.017

The University of Manchester

MANCHESTER 1824

CIGMR

Genetics of anal furunculosis in the German Shepherd Dog: Genome-wide association, replication, fine-mapping and comparative genomics

Jonathan Massey[1], Andrea Short[1], Xiayi Ke[1], UK IBD Genetics Consortium, Carl Anderson[3], William Newman[2], William ER Ollier[1], Lorna J Kennedy[1]

1 Centre for Integrated Genomic Medical Research (CIGMR), School of Medicine, University of Manchester, Manchester, M13 9PT, UK.
2 Department of Medical Genetics, University of Manchester, Manchester, M13 0JH, UK.
3 Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK.

Introduction

- Anal furunculosis (AF) is characterised by ulceration and fistulation of perianal tissue
- Particularly affects German Shepherd Dogs (GSD)
- Autoimmune component suspected due to an MHC association and clinical response to ciclosporin
- Some parallels with perianal Crohn's disease in humans

Aims

- To discover genetic risk factors for AF by Genome Wide Association Study (GWAS) and replication
- To relate findings to the human condition perianal Crohn's disease

Materials and Methods

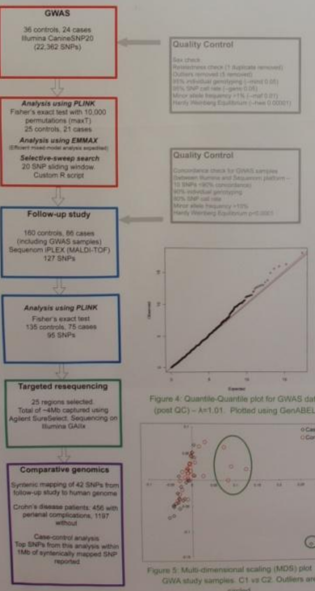


Figure 4: Quantile-Quantile plot for GWAS data (post QC) – λ=1.01. Plotted using GenABEL

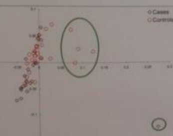


Figure 5: Multi-dimensional scaling (MDS) plot for GWA study samples. C1 vs C2. Outliers are circled.

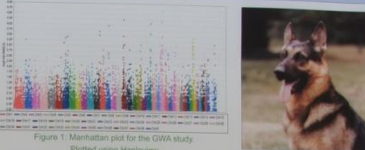


Figure 1: Manhattan plot for the GWA study. Plotted using Haploview

German Shepherd Dog

Results

- Four SNPs at 10^{-5} level in GWAS but not significant at genome-wide level
- Selective sweep (run of homozygosity) identified on Chromosome 3 (Figure 3)
- In follow-up study, seven SNPs were significant after permutation testing
  - Six SNPs on Chromosome 34 and one SNP on Chromosome 22
- Targeted sequencing underway
- 25 SNPs in comparative study with raw p value < 0.05

Figure 2: Syntenic mapping and comparative approach in Ensembl genome browser

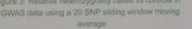


Figure 3: Relative heterozygosity cases vs controls in GWAS data using a 20 SNP sliding window moving average

Discussion

- Five of the SNPs on Chromosome 34 in a haplotype block in a gene region with plausible biological function
- One SNP in comparative study upstream of this gene – possible importance in human population

Future work

- Permutation testing on human perianal Crohn's data
- Fine-mapping causative SNPs
- Immunohistochemistry in disease vs control tissue for markers discovered from GWA and follow-up
- SNPs around the candidate gene could be included in human genotyping studies

Take home messages

- Canine GWAS with small numbers is successful in identifying novel candidate loci associated with Anal furunculosis
- Anal furunculosis may share genetic risk factors with human perianal Crohn's disease

Acknowledgements

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Глотов О.С., 2001

P09.032

Association of the ciliary gene AHI1 with autism

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Introduction

Joubert syndrome (JBTS) is an autosomaal recessive disorder with developmental delay and hypotonia, characterized by the neuroradiologic 'molar tooth sign'. Up to 27% of JBTS patients show features of autism. Thus far, 10 genes for JBTS have been identified (ref.1). The ciliary gene *AHI1* has been found mutated in 7 to 16% of cases with JBTS. Interestingly, *AHI1* was also found to be associated with autism (ref. 2). As JBTS is a ciliopathy and *AHI1* a ciliary gene, we investigated the hypothesis that autism may be partly explained by dysfunctioning of the primary cilia. We looked for association between the 10 (all ciliary) JBTS genes and autism, and determined whether a higher than expected portion of autism related genes are present in the ciliary proteome database [http://v3.ciliaproteome.org], that lists all genes with a potential role in ciliary functioning.

Methods I

A cohort of 84 patients with autism and a reference cohort consisting of 145 healthy subjects was genotyped using Infinium HumanHap300 Genotyping BeadChip SNP arrays analyses according to the protocol of the manufacturer (Illumina Inc., San Diego, CA, USA). All SNP calls for the *AHI1, NPHP1, CEP290, ARL13B, RPGRIP1L, MKS3, CC2D2A, OFD1, TMEM216*, and *INPP5E* genes were collected and the frequencies of major and minor alleles in the autism and the healthy cohorts were compared. The two-sided Fisher exact test was used to test for significance of difference.

Methods II

For selection of genes associated with autism we evaluated all genes that came up in OMIM [www.ncbi.nlm.nih.gov/omim] (version May 2010) on the keyword "autism". Of these 140 genes, we selected those that seemed to be associated with autism through at least two association studies, or one association study with a distinct non-silent polymorphism in the gene, or one association study and an animal model, or genes involved in monogenic disorders in which a diagnosis of autism was made in at least two patients, or mentioned as a feature in the clinical synopsis for that monogenic disorder. As a control sample we used a set of 100 genes that were extracted from OMIM by computerized randomisation.

References

1. Joubert syndrome and related disorders. Brancati F, et al. Orphanet J Rare Dis. 2010; 5:20.
2. Association of common variants in the Joubert gene (AHI1) with autism. Alvarez-Retuerto et al. Hum Mol Genet 2008; 17(24):3887-96.

Results I

For nine out of ten JBTS genes no significant differences in SNP allele frequencies were found between the autism and the healthy cohorts. For three SNPs mapping in and next to *AHI1* on 6q23.3 significant differences using the two-sided Fisher exact test were found. The O.R. ratios for SNP rs12179084 up to SNP rs7766656 (red marks in Fig. 1) ranged from 3.07 to 9.18, with their 95% confidence intervals excluding 1.0 (see Fig.1). We confirm the association between the ciliary gene *AHI1* and autism.

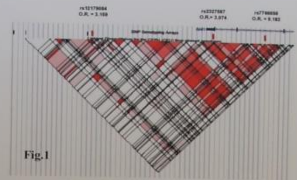

Fig.1

Results II

In Table 1 we list the 39 selected autism genes that fulfilled the criteria mentioned above and their presence in the ciliary proteome database. Twenty nine of the 39 genes, i.e. 74% (C.I. 60-88%) were present in the ciliary proteome database compared to 42 of 100 random genes (42%, C.I. 32-52%). Around forty percent is the expected baseline, as the whole genome consists of approximately twenty thousand genes, and the ciliary proteome database contains around eight thousand genes. Thus, this in silico analysis suggests that the majority of autism-related genes encode ciliary proteins.

Table 1

OMIM	Gene asociated with autism	Present in Ciliary Database
*608396	SLC9A9	+
*604569	CNTNAP2	+
+137192	GABRB3	+
+601728	PTEN	+
+173470	ITGB3	+
*300427	NLGN4	+
*300336	NLGN3	+
*300005	MECP2	-
*131310	EN2	+
*182138	SLC6A4	+
*137141	GABRA4	+
*606230	SHANK3	+
+600565	NRXN1	+
+137192	GABRB3	+
*138750	GLO1	+
*164860	MET	+
*604889	NBEA	+
*605284	TSC1	-
*191092	TSC2	+
*147870	WNT2	-
*605317	FOXP2	+
*612779	DPD	-
*142955	HOXA1	-
*606511	MARK1	+
*114205	CACNA1C	+
*610045	ALDH5A1	+
*607642	RAI1	+
*607280	CNTN4	+
*607108	PAX6	-
*300774	RAB39B	-
*608222	ADSL	-
*603667	SLC25A12	+
*609297	SEMA5A	+
*601881	RAX	+
*609978	CADPS2	+
*600029	DLX1	-
*126255	DLX2	+
*300298	UPF3B	-
*608892	CHD7	+
	39	29 +/10 -

Conclusion

Our data suggest that dysfunctioning of primary cilia may constitute an important neuropathological pathway in autism.

Глотов О.С., 2001

Genotype-by-nutrient association of common polymorphisms in obesity-related genes with food preferences and time structure of energy intake

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Introduction

Personal food preferences can either enhance or suppress the development of obesity and the selection and proportion of macronutrients in the diet seem to have a heritable component. In the present study, we therefore focused on dietary composition as a specific trait related to obesity and we determined whether genetic variations in leptin (LEP), LEP receptor (LEPR), adiponectin (ADIPOQ), IL-6 and pro-opiomelanocortin (POMC) underlie specific native food preferences and obesity-related anthropometric parameters.

Materials, Methods

A total of 409 unrelated Czech Caucasian individuals were recruited for the present study in a mass media campaign addressing the population of the south Moravia region of the Czech Republic.

Analysis of dietary intake

Dietary intake - Participants were furthermore advised to complete standardised 7-d food records. Food intake data were obtained from the study subjects and were further analysed, whereas the percentage of daily energy intake from carbohydrates, fat and protein as well as total energy and macronutrient intake were calculated using the Nutrimaster Diet Analysis software modified for the Czech population (Abbott Laboratories, Abbott Park, IL, USA).

Candidate genes

The selection of particular SNP was based on: (1) population frequency in the European Caucasian population; (2) their known or potential functional or regulatory impact on feeding behaviour or association in the case of synonymous SNP; and/or (3) a previously described association with obesity or feeding behaviour.

Genotyping was carried out for eight SNP in five genes related to the production of adipokines, control of energy homeostasis, appetite and satiety regulation: LEP (rs2167270); LEPR (rs1137101); ADIPOQ (rs2241766, b 94T/G); IL-6 (rs1800797, rs1800795); POMC (rs3754860, rs1009388).

Determination of plasma leptin, soluble leptin receptor and adiponectin

Blood samples for total LEP, ADIPOQ and sObR plasma analyses were collected after overnight fasting and were immediately centrifuged at 1700 g for 20 min and then stored at -80ºC until analysis. Plasma LEP and sObR levels were measured by commercially available sandwich ELISA (R&D Systems, Minneapolis, MN, USA) with a sensitivity of 7·8 pg/ml and 0·057 ng/ml, respectively. Plasma samples for LEP and sObR were 100-fold and 5-fold diluted with calibrator diluent immediately before the assay, respectively.

Table 1. Descriptive statistics of the patients' baseline characteristics
(Mean values and standard deviations)

Group...	Obese (n 252)				Morbidly obese (n 64)*				Controls (n 157)			
	Female		Males		Females		Males		Females		Males	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Subjects (n)	188		64		51		13		120		37	
Body composition												
Age (years)	50·1	11·4	46·4	12·2	51·6	10·6	48·7	13·0	38·8	13·2	36·8	14·0
BMI (kg/m²)	37·5	6·3	37·0	6·0	45·3	5·2	46·9	5·3	25·0	3·3	25·7	2·5
Body fat (%)	46·3	5·9	32·8	6·7	52·4	4·1	41·9	3·6	31·5	7·0	19·5	6·0
Dietary intake												
Energy (kJ)	7848	2410	10791	3201	7344	1885	10357	3394	7799	1768	10747	2425
Protein (% energy)	15·6	3·5	14·8	2·7	15·6	3·0	15·6	3·5	14·2	2·5	13·4	1·9
Carbohydrates (% energy)	49·4	5·4	49·6	5·4	49·6	5·7	48·8	4·9	51·1	5·1	51·6	6·1
Fat (% energy)	35·0	4·9	35·6	5·2	34·8	4·9	35·6	5·3	34·7	4·7	34·8	5·9
Hormonal status†												
Leptin (ng/ml)	45·6	25·8	37·3	17·7	45·1	25·6	38·1	18·5	29·6	28·1	NA	
sObR (ng/ml)	20·4	4·3	16·8	4·0	20·2	4·3	16·3	4·0	27·7	5·2	NA	
Leptin:sObR ratio	2·4	1·4	2·2	0·8	2·4	1·3	2·3	0·8	1·1	1·0	NA	
Adiponectin (μg/ml)	9·3	5·1	8·4	6·6	9·8	5·1	8·8	6·7	9·7	5·6	NA	
Anthropometry												
Waist circumference (cm)	103·9	8·9	116·0	9·2	124·5	10·2	141·9	11·2	82·1	9·7	90·1	11·6
Hip circumference (cm)	119·2	7·6	114·1	6·1	139·1	12·6	137·0	10·8	102·3	7·9	100·1	9·2
Waist:hip ratio	0·9	0·1	1·0	0·1	0·9	0·1	1·0	0·1	0·8	0·1	0·9	0·1
Skinfold thickness (mm)												
Supraspinal skinfold	26·0	7·9	23·1	8·8	30·3	12·5	32·0	16·4	19·4	24·4	15·3	5·8
Subscapular skinfold	30·1	21·6	28·4	8·7	35·4	10·0	31·0	14·6	19·1	10·6	22·4	26·9
Bicipital skinfold	22·1	6·4	16·9	5·6	26·3	8·2	25·1	8·0	14·5	5·1	11·3	6·3
Tricipital skinfold	29·6	5·6	22·9	7·0	31·5	6·9	29·1	7·4	21·5	5·5	16·5	5·3
Sum of all skinfolds	107·7	27·5	91·3	20·0	122·8	30·3	117·2	33·3	74·2	31·5	65·4	32·8
Systolic blood pressure (mmHg)	135·2	19·5	141·0	17·5	140·0	24·7	140·3	17·0	121·9	17·9	125·8	12·5
Diastolic blood pressure (mmHg)	89·0	11·0	92·3	13·6	93·1	17·8	95·7	12·8	81·7	11·1	79·7	11·2

NA, not analysed.
*Subset of obese subjects.
†Analysed in a subset of individuals, consisting of the sixty-four morbidly obese subjects and sixty-four subjects from the other groups matched by age and sex.

Table 2. Distributions of genotypes and alleles of examined polymorphisms in the studied subpopulations*

Polymorphism	Genotypes				Alleles		
ADIPOQ rs2241766 (+45T/G) (synonymous coding, GGT → GGG, Gly → Gly)	TT	TG	GG	P	T	G	P
Obese cases	149 (81)	28 (15)	7 (4)	0·36	326 (89)	42 (11)	0·42
Morbidly obese cases	51 (80)	12 (19)	1 (1)	0·90	114 (84)	14 (16)	0·64
Controls	126 (82)	25 (16)	2 (2)	–	277 (91)	29 (9)	–
ADIPOQ +94T/G (synonymous coding, GGT → GGG, Gly → Gly)	TT	TG	GG	P	T	G	P
Obese cases	97 (61)	45 (28)	16 (11)	0·26	239 (76)	77 (24)	0·74
Morbidly obese cases	35 (61)	19 (33)	3 (6)	0·96	89 (78)	25 (22)	0·79
Controls	82 (59)	48 (35)	8 (6)	–	212 (77)	64 (23)	–
LEP rs2167270 (+19A/G) (5' UTR)	AA	AG	GG	P	G	A	P
Obese cases	73 (40)	84 (46)	24 (14)	0·66	230 (63)	132 (37)	0·85
Morbidly obese cases	30 (45)	28 (42)	6 (13)	0·41	88 (69)	40 (31)	0·24
Controls	58 (37)	80 (52)	18 (11)	–	196 (63)	116 (37)	–
LEPR rs1137101 (+27265A/G) (synonymous coding, CAG → CGG, Gln → Arg)	AA	AG	GG	P	A	G	P
Obese cases	48 (26)	98 (53)	39 (21)	0·30	194 (52)	176 (48)	0·59
Morbidly obese cases	20 (31)	33 (51)	12 (18)	0·66	77 (57)	57 (43)	0·75
Controls	50 (32)	70 (45)	36 (23)	–	170 (54)	142 (46)	–
POMC rs3754860 (+1798C/T) (5' UTR)	++	+−	−−	P	+	−	P
Obese cases	76 (41)	90 (49)	19 (10)	0·68	242 (65)	128 (35)	0·40
Morbidly obese cases	23 (35)	34 (52)	8 (13)	0·34	80 (62)	50 (38)	0·16
Controls	71 (45)	73 (46)	13 (9)	–	215 (69)	99 (31)	–
POMC rs1009388 (+1032C/G) (intronic)	CC	CG	GG	P	C	G	P
Obese cases	111 (59)	64 (34)	12 (7)	0·75	286 (76)	88 (24)	0·89
Morbidly obese cases	40 (62)	21 (32)	4 (6)	0·91	101 (78)	29 (22)	0·66
Controls	96 (62)	48 (31)	12 (7)	–	240 (77)	72 (23)	–
IL-6 rs1800797 (−596A/G) (5' UTR)	GG	GA	AA	P	G	A	P
Obese cases	56 (32)	90 (51)	31 (17)	0·94	202 (57)	152 (43)	0·75
Morbidly obese cases	22 (34)	28 (44)	14 (22)	0·54	72 (56)	56 (44)	0·94
Controls	46 (30)	80 (52)	28 (18)	–	172 (56)	136 (44)	–
IL-6 rs1800795 (−174G/C) (5' UTR)	CC	CG	GG	P	C	G	P
Obese cases	54 (31)	92 (52)	30 (17)	0·88	200 (57)	152 (43)	0·67
Morbidly obese cases	20 (31)	30 (47)	14 (22)	0·82	70 (55)	58 (45)	0·93
Controls	43 (30)	75 (51)	28 (19)	–	161 (55)	131 (45)	–

ADIPOQ, adiponectin; LEP, leptin; UTR, untranslated region; LEPR, leptin receptor; POMC, pro-opiomelanocortin.
*Numbers in parentheses are the percentages of the genotypes present in the different groups. For some genotypes, only a 83–90% success rate could be reached due to a less efficient PCR amplification.

Results

Independently of the BMI of the individuals, common variations in LEP and LEPR genes were associated with specific eating patterns, mainly with respect to timing of eating. The LEP +19A/G polymorphism served as an independent predictor for BMI, percentage of body fat and skinfold thickness and significantly affected the time structure of the daily energy intake. The POMC Rsa I polymorphism was associated with percentage of body fat.

The ADIPOQ +45 T/G polymorphism was associated with the thickness of the subscapular skinfold. The LEPR Gln223Arg polymorphism was associated with multiple parameters, including diastolic blood pressure, meal sizes during the day and plasma ADIPOQ levels. In a separate analysis, soluble leptin receptor (sObR) plasma levels and LEP:sObR ratio were significantly correlated with systolic blood pressure (β = -0.66; p = 0.002; β = -1.23, p = 0.02) and sObR plasma levels also served as an independent predictor for diastolic blood pressure (β = -0.50; p = 0.04).

Discussion

To conclude, we report common allelic variants associated with specific feeding behaviour and obesity-related anthropometric traits. Moreover, we identified allelic variants that significantly influence the time structure of food intake during the day.

Table 3. Association between the upper and lower tertiles of extreme snacking behaviour with obesity in the studied cohorts

	Obesity (+)		Obesity (−)				
	%	Total n	%	Total n	OR	95 % CI	P
Total					0·57	0·35, 0·95	0·019*
Upper tertile	75	248	59	155			
Lower tertile	93	248	42	155			
Male					1·26	0·42, 3·74	0·44
Upper tertile	13	61	7	36			
Lower tertile	28	61	19	36			
Female					0·42	0·23, 0·77	0·003*
Upper tertile	62	187	52	119			
Lower tertile	65	187	23	119			

*P<0·05.

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Глотов О.С., 2001

A genome-wide association study for two autoimmune diseases in Sardinia

Centro di Ricerca, Sviluppo e Studi Superiori in Sardegna

Consiglio Nazionale delle Ricerche

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Introduction

Autoimmune diseases are extremely common in Sardinians, with the highest incidence worldwide observed for Multiple Sclerosis (MS) and Type 1 Diabetes (T1D). To detect novel loci associated with both diseases we are studying a sample of >6,000 Sardinians, including both families and unrelated individuals that were genotyped with the Affymetrix 6.0 array.

Methods

- We used birdseed-v2 algorithm to perform genotype calling over the entire sample of 6,618 Sardinians, and successively applied standard quality filters to each sample and marker.
- We selected unrelated individuals to perform 3 GWAS on MS, T1D and shared loci (MS+T1D) respectively and re-applied quality filters to each marker on each dataset.

Imputation

To increase the number of tested variants, we used the following haplotype reference panels generated from low-pass sequencing data:

- 347 Sardinian samples, sequenced at average 3x coverage using the Illumina platform.
- 280 Europeans from the 1000 Genomes Project Pilot 1 August Release.

Results and Conclusions

- We confirmed the association at some known loci (Table1).
- Imputation quality was greater using the Sardinian reference panel, especially at low frequency variants (Table 2) and a greater number of SNPs passed imputation quality filters (Figure 1, Table 2).
- Striking differences in association results were seen at HLA and *INS* gene for T1D. The Sardinian based imputation boosted power at such loci (Figure 2).

Figure 1. Schematic representation of the study design

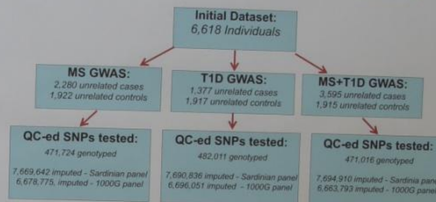
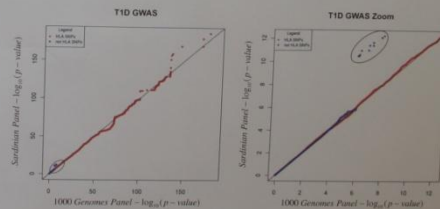


Figure 2. Comparison between p-values for imputed SNPs in T1D



Sequencing based imputation GWAS on isolated populations will benefit from using data generated on site where haplotype diversity is highly similar to the underlying LD patterns.

Table 1. Association at known loci using Sardinian and 1000G reference panels

Genes[1]	Sardinia Imputation best signals						1000 Genomes imputation best signals							
	Alleles	Freq1 Ca/Co	RSQR	OR (95% CI)	p-value	I/G[2]	Alleles	Freq1 Ca/Co	RSQR	OR (95% CI)	p-value	I/G[2]	Same SNP?	CEU r[2]
T1D														
CTLA4	A,G	0.75,0.79	0.98	0.76 (0.67-0.87)	3.96E-005	I	A,G	0.74,0.79	0.99	0.76 (0.64-0.89)	3.72E-005	I	NO	1.000
HLA	G,A	0.78,0.35	0.93	7.35 (6.28-8.59)	2.89E-186	I	A,G	0.78,0.50	0.45	16.26 (12.98-20.38)	1.59E-178	I	NO	0.657
IL2RA	T,C	0.58,0.63	0.99	0.8 (0.71-0.89)	3.45E-005	I	C,T	0.59,0.63	0.99	0.8 (0.71-0.89)	4.03E-005	I	NO	0.997
INS	G,T	0.88,0.83	0.55	2.09 (1.7-2.58)	5.53E-013	I	G,T	0.73,0.70	0.34	1.73 (1.41-2.12)	7.32E-008	I	YES	1.000
CLEC16A	C,T	0.93,0.95	0.85	0.62 (0.49-0.78)	6.65E-005	I	A,G	0.48,0.44	0.89	1.2 (1.1-1.35)	1.10E-003	I	NO	0.065
MS														
CD58	T,C	0.94,0.91	0.89	1.44 (1.2-1.7)	4.88E-005	I	C,T	0.93,0.91	0.85	1.41 (1.19-1.67)	1.80E-004	I	NO	0.935
CBLB	T,C	0.87,0.83	0.99	1.35 (1.19-1.52)	1.36E-006	G	G,A	0.87,0.83	0.99	1.34 (1.19-1.51)	2.29E-006	I	NO	1.000
HLA	G,A	0.55,0.69	0.89	0.52 (0.47-0.57)	1.06E-042	I	C,T	0.76,0.85	0.67	0.41 (0.36-0.47)	1.06E-039	I	NO	NA
IL2RA	G,T	0.58,0.52	0.94	1.3 (1.18-1.42)	1.80E-008	I	G,T	0.6,0.54	0.97	1.28 (1.17-1.4)	2.27E-009	I	YES	1.000
CLEC16A	C,T	0.58,0.64	0.99	0.81 (0.74-0.88)	2.36E-006	I	A,G	0.69,0.66	0.96	1.19 (1.08-1.3)	3.86E-004	I	NO	0.364
IRF8	C,G	0.75,0.72	0.98	1.19 (1.07-1.31)	7.07E-004	I	C,T	0.79,0.76	0.94	1.23 (1.11-1.37)	1.30E-004	I	NO	0.892
MS+T1D														
HLA	C,T	0.71,0.53	0.54	3.8 (3.38-4.27)	2.45E-125	I	T,C	0.6,0.36	0.99	2.5 (2.3-2.72)	2.25E-117	I	NO	NA
IL2RA	T,G	0.94,0.92	0.96	1.45 (1.24-1.69)	5.22E-006	I	A,T	0.94,0.92	0.94	1.47 (1.25-1.72)	2.52E-006	I	NO	1.000

Table 2. Imputation Quality

MAF	# QC-ed imputed variants Sardinian	mean RSQR Sardinian	# QC-ed imputed variants 1000G	mean RSQR 1000G
MS				
1%-3%	1,275,990	0.73	884,402	0.66
3%-5%	779,669	0.81	752,123	0.78
>5%	5,164,746	0.89	4,597,516	0.86
T1D				
1%-3%	1,286,649	0.74	888,455	0.66
3%-5%	784,118	0.81	758,727	0.78
>5%	5,161,407	0.89	4,595,500	0.86
MS+T1D				
1%-3%	1,310,389	0.73	897,000	0.66
3%-5%	773,491	0.81	754,001	0.78
>5%	5,161,001	0.89	4,654,536	0.86

References
[1] Sanna S. et al., *Nature Genetics* 2010; **42**(6):495-7
[2] The 1000 Genomes Project Consortium, *Nature* 2010; **467**:1061-1073
[3] Pilia G. et al., PLoS Genetics; **2**: e132, 2006

Notes
1) +/- 50 kb excluding HLA genes
2) I = imputed, G = genotyped

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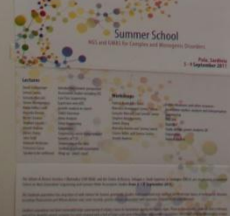


Глотов О.С., 2001

Meta-analysis of genome wide association studies reveals new loci associated with childhood obesity

H. Rob Taal[1], Jonathan P. Bradfield[2], Vincent W. V. Jaddoe[1] and Struan F.A. Grant[2,3] on behalf of the EGG consortium

1) Erasmus Medical Center, Rotterdam, Netherlands; 2) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA; 3) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

ABSTRACT

A number of genetic determinants of adult obesity have already been established through large scale meta-analyses of genome wide association studies (GWAS), several of which were also confirmed in the context of childhood obesity. However, less progress has been made to establish genetic influences specific to childhood obesity though similar approaches. To identify novel genetic factors that influence early-onset obesity, we performed a meta-analysis of genome-wide genotyped datasets from 14 study sites consisting of 5,447 cases (≥95th percentile of BMI achieved any time from age 2 to 18 years old) and 8,185 controls (<50th percentile of BMI consistent throughout all measures during childhood) of European ancestry. Following the meta-analysis of ~2.54 million SNPs (directly genotyped or imputed), variation at seven loci yielded association at the level of genome wide significance ($P < 5x10^{-8}$). All these loci have been previously reported in the context of adult BMI GWAS (*FTO*, *TMEM18*, *POMC*, *MC4R*, *FAIM2*, *TNNI3K* and *SEC16B*). *FTO* yielded the strongest evidence for association while *TNNI3K* and *POMC*, which were only detected in adult studies when using hundreds of thousands of participants, were readily detected in our relatively small sample size. We elected to take forward all novel loci yielding association with $P < 5x10^{-6}$ (n = 8) in order to test for replication in independent datasets. We observed two loci that yielded a genome wide significant *P*-value when combined with the discovery cohort, namely near keratoconus gene 6 (*KC6*) on 18q12 (rs17697518; combined $P = 9.05x10^{-10}$) and near olfactomedin 4 (*OLFM4*) on 13q14 (rs9568856; combined $P = 2.03x10^{-8}$). By also exploring association with the inclusion of extreme childhood obesity, we continued to observe a genome wide significant P-value at the locus near *OLFM4* (rs9568856; combined $P = 1.00x10^{-8}$). In summary, as a consequence of extensive North American-Australian-European collaborative meta-analyses of genome-wide genotyped datasets on children, we have uncovered at least two novel obesity loci.

INTRODUCTION

- Obesity is a major health problem in modern societies, with increasing prevalence in Western societies, particularly in children.
- There is strong evidence for a genetic component to the risk of obesity.
- In the past four years, many genetic loci have been implicated for BMI/obesity from the outcomes of GWAS, primarily in adults; however, these loci only account for a small fraction of the heritability that is known to contribute to obesity
- Distillation of the genetic component in this complex phenotype should be easier to determine in children, where environmental exposure and impact has been for a relatively short period of their lifetime
- We performed a large scale meta-analysis of 14 existing GWAS datasets for childhood obesity, totaling 5,447 cases and 8,185 controls.

MATERIALS AND METHODS

GWAS meta-analysis of childhood obesity

Discovery samples, genotyping and imputation

We selected fourteen studies with body mass index (BMI) measured in childhood (age range 2-18 years) and GWAS data available by the beginning of May 2010 (combined N= 5,447 cases / 8,185 controls): the Avon Longitudinal Study of Parents And Children (ALSPAC); The British 1958 Birth Cohort – Wellcome Trust Case Control Consortium Subset (B58C-WTCCC); The British 1958 Birth Cohort – Type 1 Diabetes Genetics Consortium subset (B58C-T1DGC); the Cardiovascular Risk in Young Finns Study (YF); The Children's Hospital of Philadelphia (CHOP); the Copenhagen Study on Asthma in Childhood (COPSAC); The Generation R Study (GENR); The Helsinki Birth Cohort Study (HBCS); the Lifestyle – Immune System – Allergy Study (LISA); the Northern Finland 1966 Birth Cohort (NFBC1966); The Raine study (RAINE); the GOYA study (GOYA); the French Young study (FRENCH YOUNG) and the Essen Study (ESSEN). Cases were defined as having a BMI>95th percentile at any point in childhood. Controls were defined as consistently having a BMI<50th percentile throughout childhood for all measurements available for that individual. BMI percentiles were based on national standard growth curves, except in the Helsinki Birth Cohort Study (HBCS) as pediatric measurements were made two decades ago, thus contemporary curves will not be appropriate. HBCS generated their own reference curves. Known syndromic cases of obesity were excluded, since these individuals are likely to have a different underlying genetic architecture. We used principal components analysis in order to minimize the potential impact of population stratification in the CHOP, ESSEN and FRENCH YOUNG sample sets. Eigenstrat 3.0 was employed to remove outliers and to subsequently calculate the principal components. The principal components were then used as covariates in a logistic regression, using the software mach2dat, to compute the *P*-values, odds ratios and standard errors.

Statistical analysis within discovery samples

Genotypes were obtained using high-density SNP arrays, and then imputed for ~2.4 million HapMap SNPs (Phase II, release 21/22). Informed consent was obtained from all discovery study participants (or parental consent, as appropriate), and study protocols were approved by the local ethics committees. The association between each SNP and case-control status was assessed in each study sample using logistic regression of case-control against genotype, assuming an additive model. Imputed genotypes were only used where directly-assayed genotypes were unavailable.

Meta-analysis of discovery samples

Prior to meta-analysis, SNPs with a minor allele frequency <1% and poorly-imputed SNPs were filtered. Fixed effects meta-analyses were conducted by two independent investigators. Meta-analysis was performed using the software package METAL; Genomic control was applied twice at the meta-analysis stage: first, to adjust the statistics generated within each cohort and second, to adjust the overall meta-analysis statistics. Meta-analysis was carried using the inverse-variance method, fixed effects model was assumed. SNPs available for less than half of the total expected sample were excluded.

Follow-up of lead signals in additional samples

Follow-up samples, genotyping and analysis

We used 10 study samples to follow up the 8 novel signals from the GWAS meta-analysis. Informed consent was obtained from all follow-up study participants (or parental consent, as appropriate), and study protocols were approved by the local ethics committees. If the index SNP was unavailable, a closely correlated proxy was substituted.

Meta-analyses

We performed fixed effects inverse variance meta-analyses of the association results for the 8 lead signals in the 14 discovery samples and 10 replication samples combined. Fixed effects meta-analyses were again conducted independently by two investigators, using the software package METAL. We used the Cochran Q test and the *I2* statistic to assess evidence of between-study heterogeneity of effect sizes.

Sensitivity analysis

Two of the replication samples are comprised of extremely obese children. Since these are more extremely obese than the discovery samples and the remaining three replication samples, it could be they have a different underlying genetic make-up. We performed a sensitivity analysis by leaving these two cohorts out of the replication effort.

EGG CONSORTIUM CHILDHOOD OBESITY STUDY GROUP (Alphabetical)

I. Barroso, R.I. Berkowitz, H. Bisgaard, D.I. Boomsma, J.P. Bradfield, D.L. Cousminer, I.S. Farooqi, P. Froguel, F. Gilliland, M.W. Gillman, S.F.A. Grant, M. Guxens, H. Hakonarson, J. Hebebrand, J. Heinrich, A. Hinney, A. Hofman, C. Holst, E. Hypponen, V.W.V. Jaddoe, M.R. Jarvelin, C. Lecoeur, M.I. McCarthy, A. Meirhaeghe, D. Meyre, D.O. Mook-Kanamori, S. O'Rahilly, L.J. Palmer, C. Power, F. Rivadeinera, R.M. Salem, V. Santhanakrishnan, A. Scherag, F. Schumacher, P.M.A. Sleiman, G.D. Smith, T.I.A. Sørensen, H.R. Taal, E. Thiering, N.J. Timpson, A.G. Uitterlinden, B. Valcarcel, R.J.P. van der Valk, C.M. van Duijn, N. Warrington, E. Wheeler, E. Widen, J. Zhao

RESULTS

- Following the meta-analysis of ~2.54 million SNPs, variation at seven loci revealed genome wide significance levels for association with childhood obesity ($P < 5.0x10^{-8}$) – Table 1
- Took forward all novel loci yielding association with $P < 5.0 x10^{-6}$ (n = 8) - Table 2
- In our replication effort, we tested these 8 SNPs in 8 cohorts that had a comparable set of patients; we observed two loci that yielded a genome wide significant *P*-value when combined with the discovery cohort, namely near keratoconus gene 6 (*KC6*) on 18q12 (rs17697518; combined $P = 9.05x10^{-10}$) and near olfactomedin 4 (*OLFM4*) on 13q14 (rs9568856; combined $P = 2.03x10^{-8}$) – Table 3
- We also had two very extreme childhood obesity cohorts available to query. By exploring association with the inclusion of these cohorts, we continued to observe a genome wide significant *P*-value at the locus near *OLFM4* (rs9568856; combined $P = 1.00x10^{-8}$) – Table 4

TABLES

Table 1: Top discovery signals at each locus (all known) that reach genome-wide significance ($P < 5x10^{-8}$) sorted by *P*-value

SNP	Chr	Pos	Allele1	Allele2	Freq1	Effect	Se	P-value	Direction	Nearest Gene
[illegible]	16	[illegible]	T	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	FTO
[illegible]	2	[illegible]	T	G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	TMEM18
[illegible]	2	[illegible]	A	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	POMC
[illegible]	18	[illegible]	A	G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	MC4R
[illegible]	12	[illegible]	A	G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	FAIM2
[illegible]	1	[illegible]	C	G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	TNNI3K
[illegible]	1	[illegible]	T	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	SEC16B

Table 2: Discovery signals at each locus that did not reach genome-wide-significance but yielded $P < 5x10^{-6}$, sorted by *P*-value

SNP	Chr	Pos	Allele1	Allele2	Freq1	Effect	Se	P-value	Direction	Nearest Gene
[illegible]	4	[illegible]	T	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	GNPDA2*
rs9568856	13	[illegible]	A	G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	OLFM4
[illegible]	4	[illegible]	T	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	BC041448
rs17697518	18	[illegible]	T	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	KC6
[illegible]	17	[illegible]	T	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	HOXB5
[illegible]	5	[illegible]	T	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	MAST4
[illegible]	4	[illegible]	A	C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	ALPK1
[illegible]	10	[illegible]	A	G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	1	[illegible]	A	G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	MTOR-ANGPTL7

*Previously reported

Table 3: Replication effort of the 8 loci taken forward in to 8 comparable independent cohorts, sorted by chromosomal location. Both separate replication data and combined data are shown

				Comparable replication samples				Combined with Discovery		
SNP	Chr	Pos	Nearest Gene	Effect	Se	P-value	Direction	Effect	Se	P-value
[illegible]	1	[illegible]	MTOR-ANGPTL7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	4	[illegible]	ALPK1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	4	[illegible]	BC041448	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	5	[illegible]	MAST4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
rs9568856	13	[illegible]	OLFM4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	17	[illegible]	HOXB5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
rs17697518	18	[illegible]	KC6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 4: Replication effort of the 8 loci taken forward in to two extreme independent cohorts, sorted by chromosomal location. Separate replication data and combined data are shown along with an overall assessment of all data used in the study

				Extreme obesity replication samples				Combined with Discovery			Meta-analysis of all discovery and replication cohorts		
SNP	Chr	Pos	Nearest Gene	Effect	Se	P-value	Direction	Effect	Se	P-value	Effect	Se	P-value
[illegible]	1	[illegible]	MTOR-ANGPTL7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	4	[illegible]	ALPK1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	4	[illegible]	BC041448	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	5	[illegible]	MAST4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
rs9568856	13	[illegible]	OLFM4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	17	[illegible]	HOXB5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
rs17697518	18	[illegible]	KC6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

CONCLUSIONS

We have isolated genetic variants that predispose to childhood obesity, which may go on to impact risk of developing type 2 diabetes and other diseases later in life. Further functional characterization of these signals is required to elucidate the precise mechanism behind these observations.

Глотов О.С., 2001

DISEASE GENE IDENTIFICATION BY EXOME SEQUENCING

Gilissen C, Hoischen A, Vissers LELM, van Bon BWM, Arts HH, de Ligt J, Rosario M, Janssen I, Arts P, van Lier B, Steehouwer M, de Vries P, Wieskamp N, de Vries BBA, Brunner HG, Veltman JA

Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

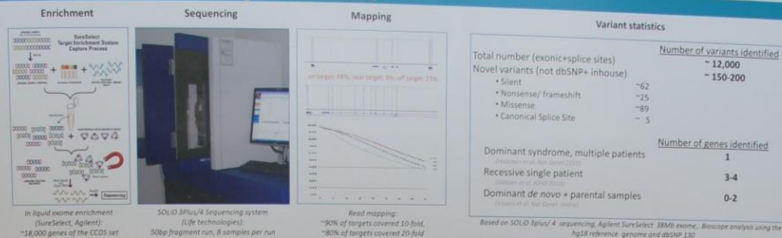
UMC St Radboud

Abstract

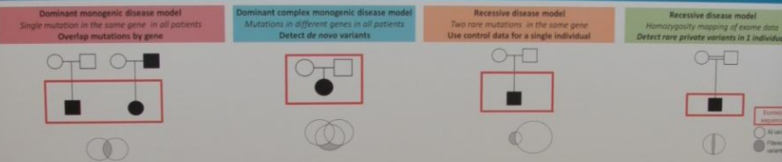
To date more than 2,500 Mendelian disease genes are known, many of which were identified through positional cloning followed by Sanger sequencing. These approaches however suffer from a limited resolution in identifying disease gene loci and are usually supplemented with a candidate gene approach. In addition, these approaches cannot be applied in most disorders that occur because of spontaneous "*de novo*" mutations in the germline. The advancement of whole exome and whole genome sequencing approaches has given a new impulse to the field by eliminating the need for positional cloning and allowing the unbiased identification of all genomic variation in a single experiment. The challenge no longer lies with selecting candidate genes, but rather with selecting candidate pathogenic variants among all the variation identified.

Here we applied whole exome sequencing followed by three different disease gene identification and prioritization strategies, taking into account the inheritance model and genetic complexity of the disease.

Exome Sequencing – Technical details



Prioritization schemes



Variants	Patient 1	Patient 2	Patient 3	Patient 4	Candidate genes
Total called	22,916	22,602	22,152	19,528	4,735
Exonic + SpliceSites(SS)	12,196	12,255	11,796	10,498	3,331
Non-synonymous (NS) + SS	5,556	5,618	5,427	4,802	1,634
New (dbSNP130 & in-house exomes)	**180**	**186**	**154**	**172**	**1**

Modified from: Hoischen et al. Nat Genet 42: 483-5 (2010)

Variants	Average of 10 patients
Total called	21,755
Non-synonymous + SpliceSites (SS)	5,640
New (dbSNP130 & in-house exomes)	143
After exclusion inherited variants	5
Validation Sanger sequencing	**0-2**

Modified from: Vissers et al. Nat Genet 42: 1109-12 (2010)

Variants/genes	Patient 1	Patient 2
Total called	22,723	22,094
Exonic + SpliceSites(SS)	12,243	13,230
Non-synonymous (NS) + SS	5,559	5,488
New (dbSNP130 & in-house exomes)	169	211
Candidate **genes** recessive disease (comp. het./ hom.)	3/0	4/0
Segregation analysis & functional evidence **(genes)**	1	1

Modified from: Gilissen et al. Am J Hum Genet. 87:418-23 (2010)

Variants	Patient
All variants	26,922
Exonic + SpliceSites(SS) (>5 variant reads, >15% variation)	13,491
Non-synonymous (NS) + SS	6,299
New (dbSNP130 & in-house exomes)	318
Homozygous variants (of which autosomal)	20 (17)
Overlapping homozygous regions	3

Modified from: Becker et al. Am J Hum Genet. 88(3):362-71 (2011)

Ongoing work

Although these approaches have been applied successfully in the rapid identification of candidate disease genes many challenges remain. The current methods of providing evidence for pathogenicity need to be adapted appropriately to deal with the higher throughput of next-generation sequencing studies. Causality is traditionally shown by:

1. **Identifying recurrent mutations in multiple patients with the same phenotype, and absence in unaffected individuals.** This requires new techniques that allow the rapid follow-up screening of a novel candidate disease gene in thousands of patients and controls.

2. **Providing functional (predictive) evidence that links the mutated gene to the disease phenotype.** This requires high-throughput functional assays to rapidly screen genetic variation for functional consequences.

Contact: c.gilissen@antrg.umcn.nl; www.genomicdisorders.nl; www.humangenetics.nl

Глотов О.С., 2001

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Development of an exome sequencing workflow in a diagnostic setting

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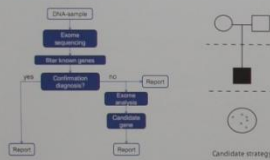
Department of Human Genetics, Radboud University Nijmegen Medical Centre, The Netherlands

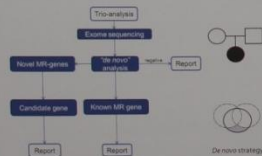
Introduction

Targeted Next Generation Sequencing approaches allow rapid and affordable analysis of genetic variation at multiple loci in parallel. This has been of enormous value in recent disease gene identification studies, especially when expanded to the exome. These results demonstrate that exome sequencing is becoming rapidly a robust approach for identification of genetic variation. Here we present the implementation of exome sequencing in a diagnostic setting.

Strategies

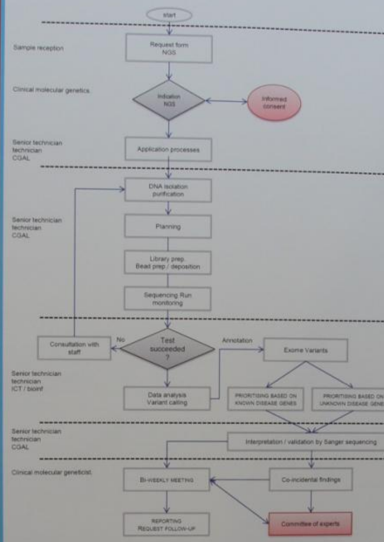


Creating gene packages allows analysis of only those genes known to cause a genetic disorder, rather than all 20,000 genes, in the first instance. If no gene alteration is identified, the remaining genes will then be analysed.



Unexplained mental retardation has a specific approach. There are few known genes, and genes are known to have a very low mutation frequency. The method is based on the assumption that "de novo" mutations are a major cause of mental retardation (*Vissers LE et al. Nat Genet. (2010)*).

Workflow



CGAL: central genome analysis laboratory

Informed consent

All individuals who qualify for exome sequencing must sign a dedicated informed consent form. The individual or legal guardian needs to be informed about the entire procedure since the data of all exomes will be stored in a database and can be used to improve diagnostic interpretation. Furthermore, they need to understand that gene alterations could be identified by chance that are not related to the disorder being investigated (co-incidental findings).

Sample traceability

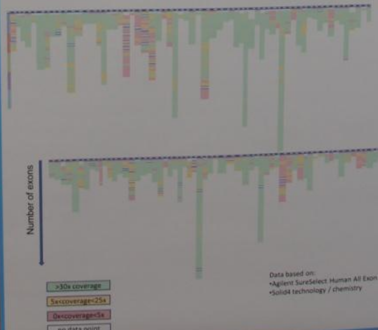
During lab procedures samples and plates have a barcode for traceability. DNA of all samples will be sequence tagged before pooling (during library prep). Using an independent test all samples will be genotyped for 90 exonic SNPs in order to exclude a sample swap.

Co-incidental findings

Any co-incidental findings will be assessed by an independent committee of experts to determine if they need to be reported to the referring clinician. In exceptional cases, the committee may decide that it is in the patient's best interest to inform.

Gene package: average coverage per exon of 144 genes

(32 exomes)



For heterogeneous genetic diseases like hereditary blindness, hereditary deafness, OXPHOS disorders, movement disorders, and bowel cancer we will first analyze / prioritize variants in known disease genes. To be able to reliably detect variants, a sequence depth of 30x coverage per exon is needed. Before interpretation of the data it is important to analyse the overall performance of a given gene package. The gene packages show a non-random distribution of poor performing exons. It is to be expected that performance will improve in time with improved technology and chemistry on a 5500XL.

Contact

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Functional genomics in individuals: Understanding biology using intra-species comparisons.

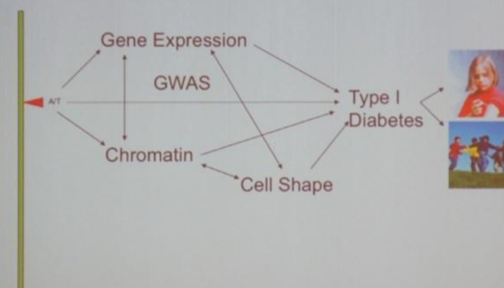
E. Birney;

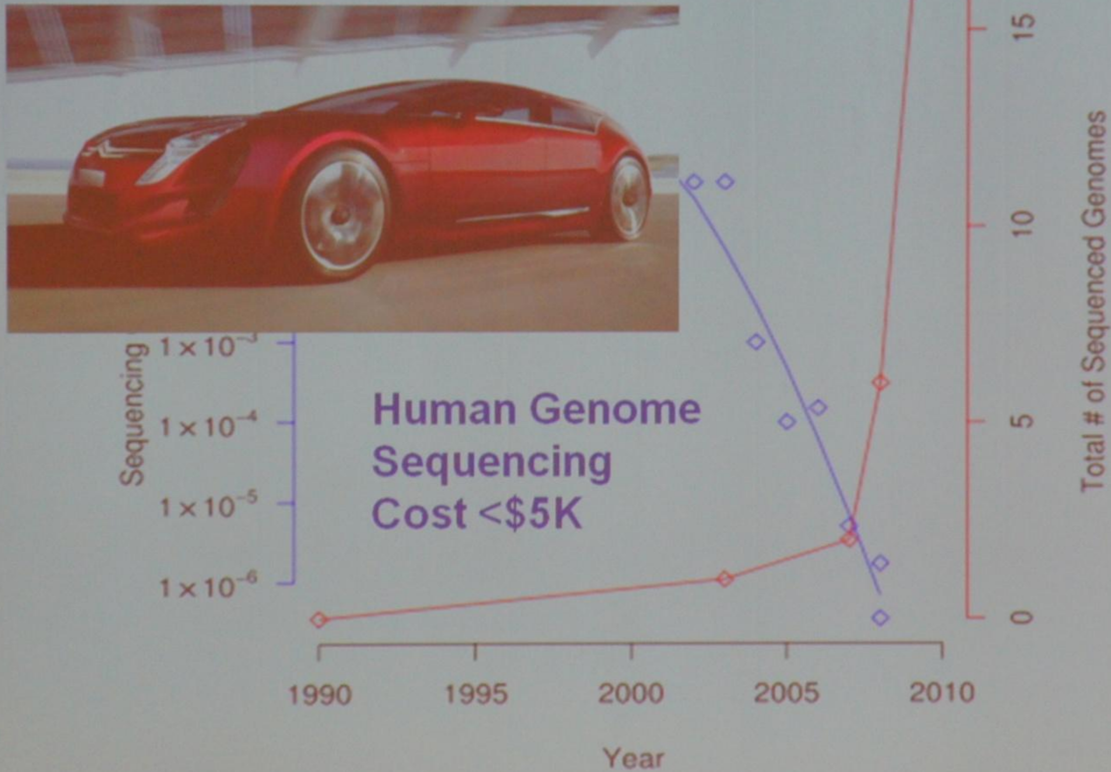
European Bioinformatics Institute, Wellcome Trust Genome Campus, EMBL Outstation - Hinxton,, Cambridge, United Kingdom.

Gene Sets

- Human and Mouse
  - Careful combination of manually reviewed gene structures and automatic, asymptoping to 100% manual review
- Non-coding RNA
  - Rfam based (structural) and Histone+Transcript based (lincRNAs)
- Other species
  - If you have cDNA resources, cDNA first
  - Then mainly protein coding homology based
  - Very consistent. Errors usually in assembly rather than algorithms
- RNAseq
  - How to do gene prediction
  - Will aim to roll this out over the next couple of years across many (perhaps all?) species

EMBL-EBI





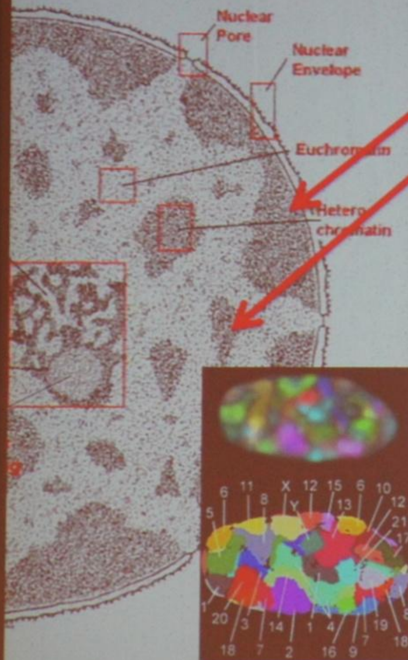
Глотов О.С., 2001

Chromatin as a key regulator of DNA repair – implications for the aging epigenome

Philipp Oberdoerffer
Mouse Cancer Genetics Program
NCI-Frederick

The aging nucleus

Phenotypic changes in aged nuclei



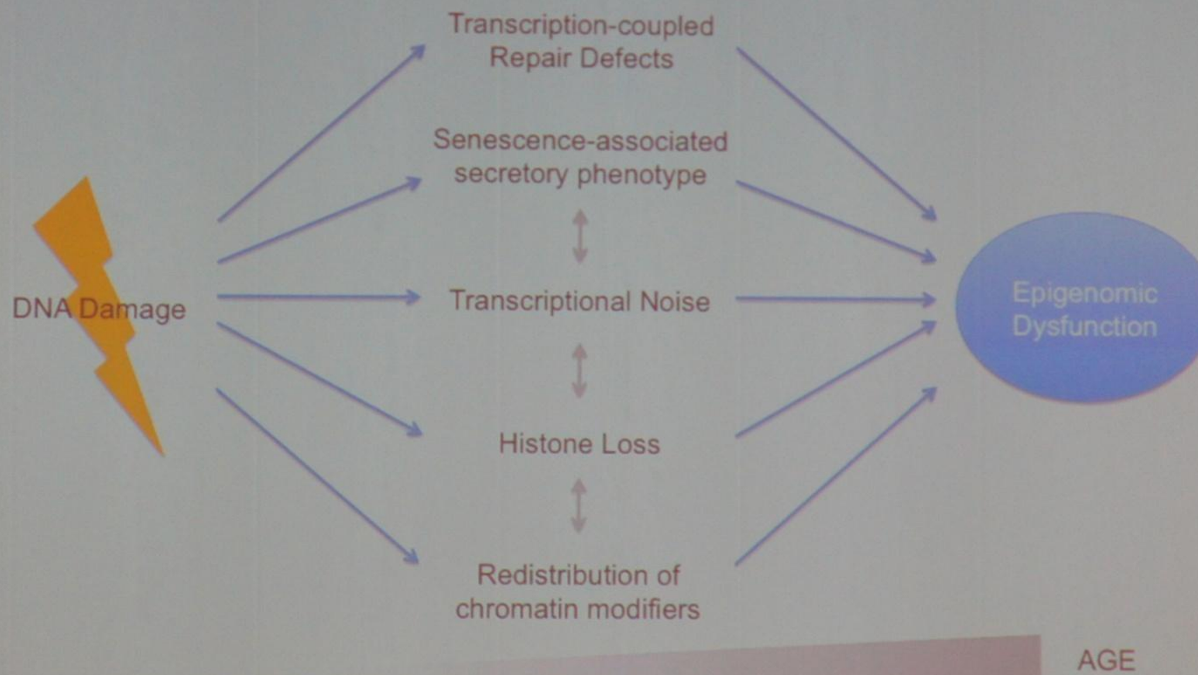
- Loss of perinuclear heterochromatin
- Senescence-associated heterochromatin foci?
- Increased DNA damage

Functional (epigenomic) changes in aged nuclei

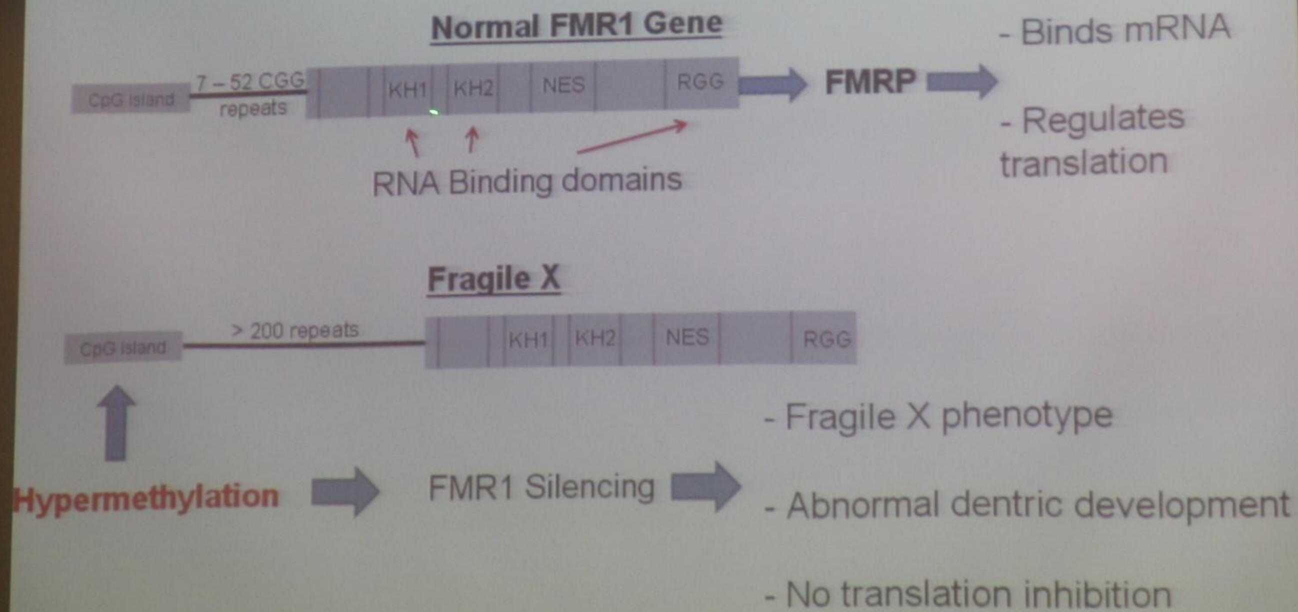
- Global age-related gene deregulation
  - → Repression of tumor suppressors
  - → altered tissue homeostasis and cell survival?

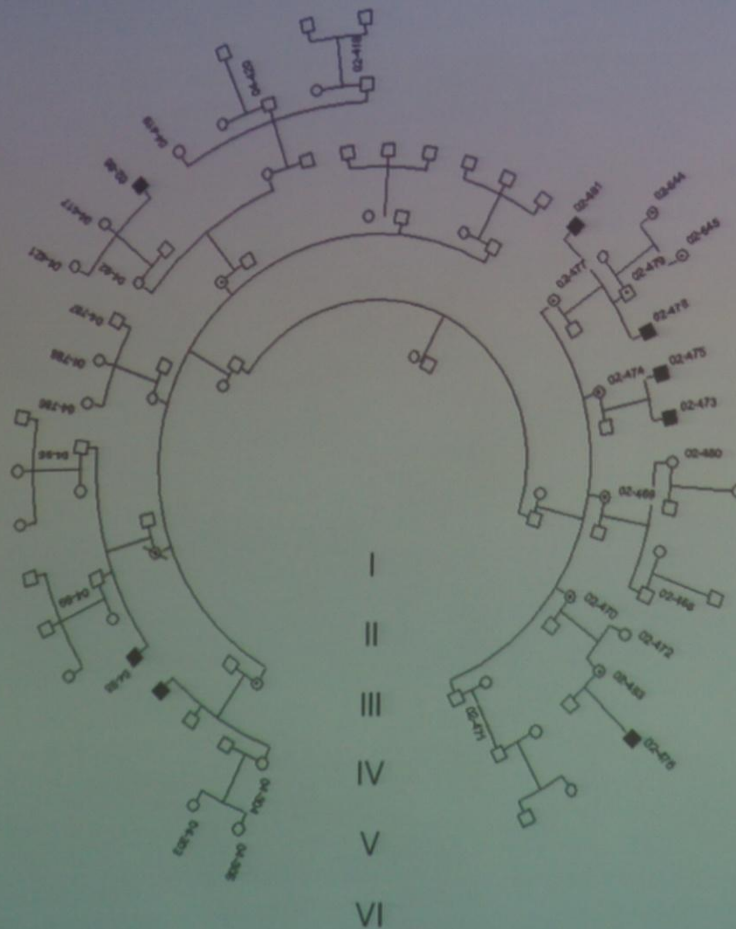
Bolzer et al., *PLoS Biol*, 2005

Глотов О.С., 2001



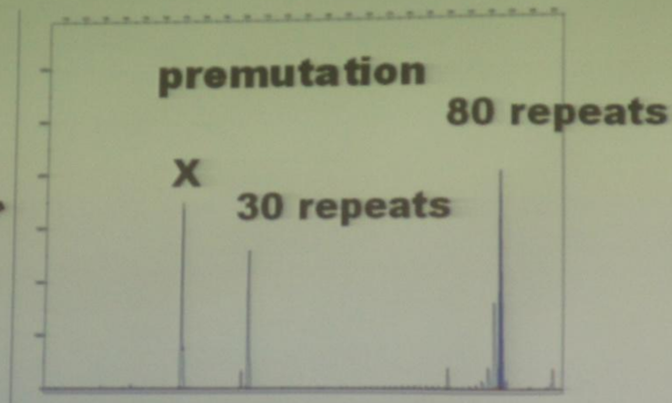
Глотов О.С., 2001

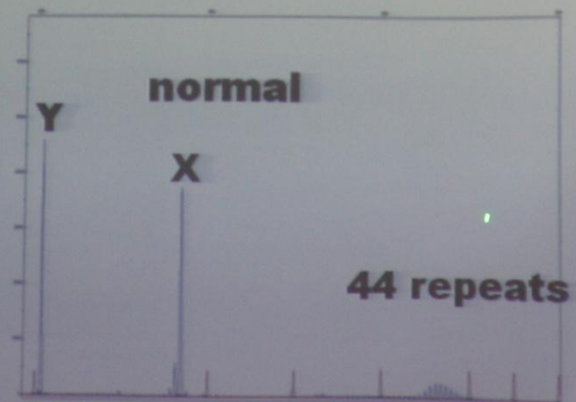

Molecular biology of fragile X syndrome (FXS)
Physiopathology
Normal FMR1 Gene
CpG island
7 – 52 CGG repeats
KH1
KH2
NES
RGG
FMRP
- Binds mRNA
- Regulates translation
RNA Binding domains
Fragile X
CpG island
> 200 repeats
KH1
KH2
NES
RGG
Hypermethylation
FMR1 Silencing
- Fragile X phenotype
- Abnormal dentric development
- No translation inhibition



Son Espases

Глотов О.С., 2001





Regression to a normal allele

Son Espases
hospital universitari

COMPARISON OF PCR AND SOUTHERN BLOTTING WITH THE ABBOTT ASR SIZING ASSAY

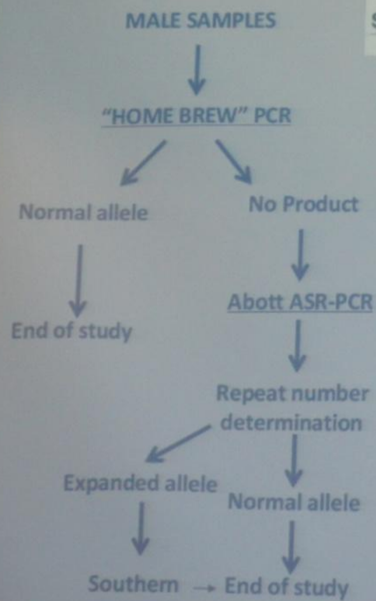
SIZE AND METHYLATION STATUS DETERMINATION

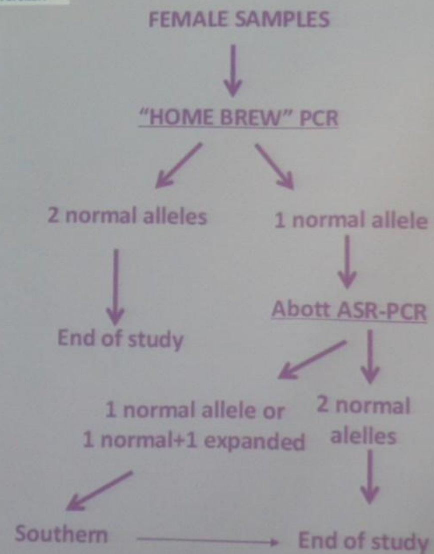
	NORMAL (6-55)	PREMUTATION (55-200)	FULL MUTATION (>200)	MOSAICISM PREMUTATION/ FULL MUTATION	DIFFERENTIATES FEMALE HOMOZYGOTES FROM CARRIERS?	METHYLATION STATUS
SOUTHERN	YES	YES	YES	YES	YES	YES
"HOME BREW" PCR (GC-RICH ASSAY, DEAZA-GTP, etc.)	YES	YES, until aprox 80 REPEATS	NO	NO	NO	NO
ABBOTT ASSAY ASR SIZING	YES	YES	YES (some times difficult)	YES	YES?	NO

OTHER CONSIDERATIONS

	PRECISION	LABOR	TIME	PRICE
SOUTHERN	IMPRECISE	INTENSIVE	3-5 DAYS	€€
"HOME BREW" PCR (GC-RICH ASSAY, DEAZA-GTP, etc.)	UNDERESTIMATES REPEAT NUMBER	NON INTENSIVE	<24 HOURS	€
ABBOTT ASSAY ASR SIZING	YES	NON INTENSIVE	<24 HOURS	€€€

WORKFLOW WITH THE ASR-PCR SIZING ASSAY





Глотов О.С., 2001

European Conference of Human Genetics 2011

Amsterdam, The Netherlands

A Genome-Wide Association Study identifies 2 loci associated with heart failure due to dilated cardiomyopathy

Inserm

Institut national de la santé et de la recherche médicale

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

UPMC

PARIS

Глотов О.С., 2001

Results after discovery phase on 517 382 SNPs

Retested SNP	Chr	Position	Locus	Pool–GWAS[a]			Individual GWAS[b]			MAF[c] cases/controls
				OR	95% IC	P-value	OR	95% IC	P-value	
rs16983785	21	29447289	TAK1L	1.97	1.60–2.42	5.4×10^{-11}	1.79	1.41–2.27	7.4×10^{-7}	0.102/0.059
rs7328410	13	104824202	Intergenic	1.73	1.45–2.06	3.2×10^{-10}	1.35	1.01–1.80	$P = 0.037$	0.056/0.045
rs2234962	10	121419623	BAG3	0.64	0.55–0.74	8.8×10^{-10}	0.53	0.44–0.63	1.1×10^{-13}	0.125/0.208
rs1991914	4	4260014	OTOP1	0.59	0.49–0.71	1.3×10^{-8}	0.99	0.87–1.13	0.9	0.339/0.338
rs13176432	5	114369723	Intergenic	0.56	0.45–0.68	1.5×10^{-8}	0.85	0.73–0.99	0.0013	0.047/0.027
rs10491858	9	1500495	Intergenic	0.68	0.59–0.80	5.8×10^{-8}	0.81	0.69–0.95	0.0049	0.140/0.111
rs5970164	X	150849762	MAGEA4	0.61	0.50–0.73	7.2×10^{-8}	0.73	0.64–0.84	4.9×10^{-6}	0.103/0.167
rs856003	10	119393553	Intergenic	0.69	0.60–0.79	1.1×10^{-7}	0.79	0.67–0.93	8.9×10^{-4}	0.195/0.157
rs10927875	1	16171899	ZBTB17	0.71	0.63–0.81	1.3×10^{-7}	0.71	0.62–0.81	3.6×10^{-7}	0.269/0.341
rs11543052	21	46880804	PRMT2	2.18	1.63–2.93	9.7×10^{-8}	2.46	1.37–4.42	0.0021	0.018/0.006
rs1353456	X	78829377	Intergenic	1.38	1.22–1.57	2.7×10^{-7}	1.25	1.11–1.40	1.2×10^{-4}	0.218/0.159
rs2832070	21	29059787	AF131217.1	1.43	1.24–1.65	3.2×10^{-7}	1.44	1.21–1.73	4.7×10^{-5}	0.155/0.111
rs1378796	3	158614482	VEPH1	1.49	1.28–1.75	3.9×10^{-7}	1.49	1.17–1.88	7.4×10^{-4}	0.099/0.074
rs2290906	17	73605461	TNRC6C	1.45	1.25–1.67	3.4×10^{-7}	1.42	1.19–1.69	5.8×10^{-5}	0.164/0.124

[a]Computed from pooled DNA signals using a linear mixed model adjusting for population, age category, and gender.
[b]Computed from individual genotypes using a logistic regression model adjusting for population, age category, and gender.
[c]Minor allele frequency estimated from individual genotypes.

Replication phase

SNP	Locus	Discovery sample Individual genotyping			Replication sample Individual genotyping		
		OR	95% CI	P-value	OR	95% CI	P-value
rs16983785	TAK1L	1.79	1.41-2.27	7.4×10^{-7}	1.25	0.98-1.61	0.076
rs2234962	BAG3	0.53	0.44 - 0.63	**1.1×10^{-13}**	0.82	0.70-0.95	**0.0092**
rs10927875	ZBTB17	0.71	0.63-0.81	**1.3×10^{-7}**	0.82	0.72-0.93	**0.0021**

Summary/Conclusion

✓**DNA pools-based GWAS** successfully identify two loci strongly associated with DCM in humans:

- One locus encompass 5 genes in strong LD on Chromosome 1 with $p = 9.5 \times 10^{-10}$ for association in combined discovery and replication samples

- Two nsSNPs in BAG3 display independent association with DCM with $p = 3.1 \times 10^{-12}$ and $p = 3.6 \times 10^{-3}$ defining a protective haplotype

✓**Mutation screening in familial DCM (n= 168)** identified 6 heterozygous mutations in BAG3 (4 early stops, 2 substitutions) absent from control population, with suspected strong functional effect and segregating in families. This is strongly supporting that BAG3 is a new morbid gene responsible for familial dilated cardiomyopathy

European Heart Journal
doi:10.1093/eurheartj/ehr105

FASTTRACK CLINICAL

A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy

Eric Villard[1,2*], Claire Perret[3], Françoise Gary[1,2], Carole Proust[3], Gilles Dilanian[1,2], Christian Hengstenberg[4], Volker Ruppert[5], Eloisa Arbustini[6], Thomas Wichter[7‡], Marine Germain[3], Olivier Dubourg[8], Luigi Tavazzi[9], Marie-Claude Aumont[10], Pascal DeGroote[11], Laurent Fauchier[12], Jean-Noël Trochu[13,14], Pierre Gibelin[15], Jean-François Aupetit[16], Klaus Stark[4], Jeanette Erdmann[17], Roland Hetzer[18], Angharad M. Roberts[19], Paul J.R. Barton[20,21], Vera Regitz-Zagrosek[22], Cardiogenics Consortium[§], Uzma Aslam[1,2], Laëtitia Duboscq-Bidot[1,2], Matthias Meyborg[7], Bernhard Maisch[5], Hugo Madeira[23], Anders Waldenström[24], Enrique Galve[25], John G. Cleland[26], Richard Dorent[27], Gerard Roizes[28], Tanja Zeller[29], Stefan Blankenberg[29], Alison H. Goodall[30], Stuart Cook[19,20], David A. Tregouet[3], Laurence Tiret[3], Richard Isnard[1,2], Michel Komajda[1,2], Philippe Charron[1,2†], and François Cambien[2,31†]

Глотов О.С., 2001

Global Burden of Type 2 Diabetes

- The prevalence of type 2 diabetes has been increasing at epidemic proportions globally
  - In 2000, the global prevalence of diabetes was 171 million, this is projected to reach 366 million by 2030
  - Worldwide, almost 3 million deaths per year are attributable to diabetes
- Diabetes leads to other chronic complications
  - It is the leading cause of kidney failure, lower limb amputations and new cases of blindness amongst US adults
  - Diabetes is the major cause of heart disease and stroke
- Diabetes also poses a major economic burden worldwide
  - In 2007, diabetes cost the United States ~ $174 billion

San Antonio Family Heart Study

- San Antonio Family Heart Study designed in 1991 to investigate the genetics of CVD in Mexican Americans
- Included 1,431 individuals from 42 families
- Rich genomic data
  - Genome-wide SNP genotypes in 1,431 individuals using the Illumina Human 1M-Duo BeadChip
  - Genome-wide transcriptional profiling of 1,240 individuals at baseline, detecting more than 22,000 transcripts
- Rich phenotypic data
  - anthropometry, blood pressure, lipids, lipidomic species, obesity, diabetes, inflammation, oxidative stress, hormones, osteoporosis, brain structure and function

Глотов О.С., 2001

Metabolomics Laboratory (Baker IDI)

Analytical platform = Liquid chromatography, electrospray ionisation triple quadrapole mass spectrometer (LC ESI-MS/MS)

LDL, HDL, Triglycerides and Cholesterol

- Ceramides
- Sphingolipidis
- Glycosphingolipids
- Phospholipids
- Di- and Triacylglyerols
- Cholesterol esters
- Modified lipids
  - oxidised, glycated
- Lysolipids
- Free fatty acids

> 10,000 different lipids in a cell (most will be in plasma at some level)

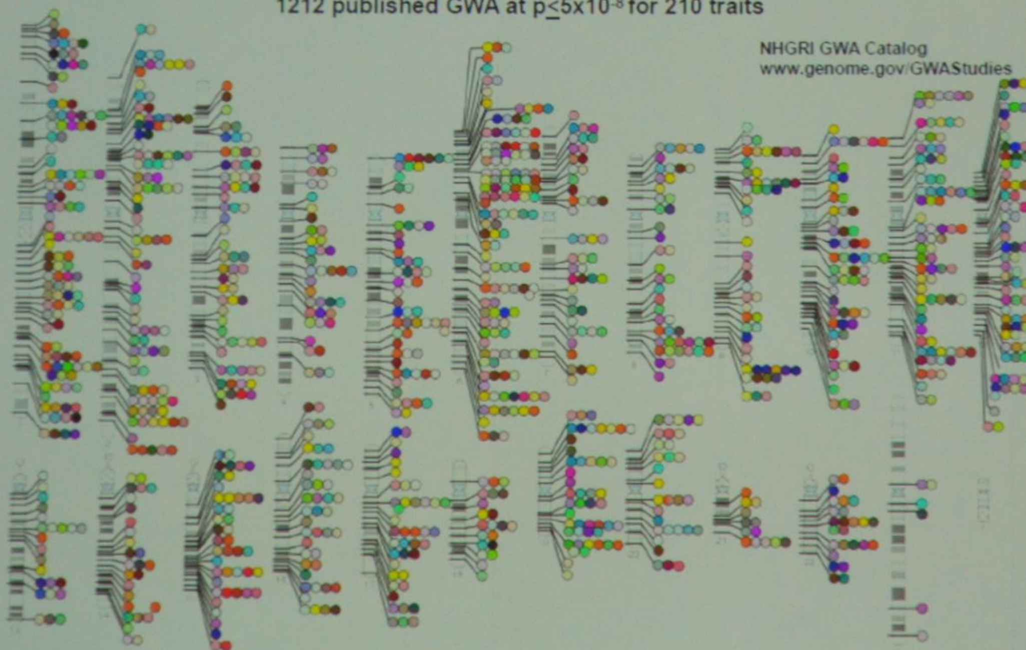
Глотов О.С., 2001

Genetic Components of Lipid Species

- 1,202 San Antonio Family Heart Study samples
  - From 40 large extended pedigrees
  - 861 individuals without diabetes (baseline prevalence 28.4%)
  - 110 (12.8%) individuals developed diabetes over 10 year follow-up
- Measured 356 lipid species
- Evidence of significant heritability for the majority of these traits
  - 349/356 lipids showed significant genetic component
  - Average $h^2 = 0.346$
  - Maximum $h^2 = 0.613$ ($p < 0.00001$) for DHC 24:1

Click on Comment and Share to create, mark-up and send PDF files.

GWAS has driven the identification of common genetic variation and association with complex traits



Глотов О.С., 2001